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(57) Abstract

Mycobacterium tuberculosis genes encoding five immunologically relevant proteins have been isolated by systematically screening a lambda gt11 recombinant DNA expression library with a collection of murine monoclonal antibodies directed against protein antigens of this pathogen. One of the *M. tuberculosis* antigens, a 65kD protein, has been shown to have determinants common to *M. tuberculosis* and *M. leprae*. In addition, genes encoding proteins of other mycobacteria (*M. africanum, M. smegmatis, M. bovis* BCG and *M. avium*) have been isolated. Isolation and characterization of genes encoding major protein antigens of *M. tuberculosis* make it possible to develop reagents useful in the diagnosis, prevention and treatment of tuberculosis. They can be used, for example, in the development of skin tests, serodiagnostic tests and vaccines specific for tuberculosis.

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MYCOBACTERIUM TUBERCULOSIS GENES AND ENCODING PROTEIN ANTIGENS

Description

Background

Tuberculosis was the major cause of infectious mortality in Europe and the United States in the 19th and early 20th centuries. Dubos, R. and J. Dubos, The White Plague: Tuberculosis, Man and Society, Little Brown & Co., Boston, MA, (1952). Today, it remains a significant global health problem.

For example, in the United States there are over 20,000 new cases of tuberculosis diagnosed annually. In addition, the steadily declining incidence of tuberculosis evident in preceding years appears to have changed course, reaching a plateau in 1985 and showing an increase in the first half of 1986. Centers for Disease Control, Morbidity/Mortality, Weekly Report, 34:774 (1986); and Centers for Disease Control, Morbidity/Mortality, Weekly Report, 35:774 (1986).

Worldwide, tuberculosis remains widespread and constitutes a health problem of major proportions, particularly in developing countries. The World Health Organization estimates that there are ten million new cases of active tuberculosis per year and an annual mortality of approximately three

million. Joint International Union Against Tuberculosis and World Health Organization Study Group, Tubercle, 63:157-169 (1982).

Tuberculosis is caused by Mycobacterium (M.) tuberculosis or Mycobacterium (M.) bovis, which are the 'tubercle bacilli' of the family Mycobacteriaceae. M. bovis is a species which causes tuberculosis in cattle and is transmissible to humans and other animals, in whom it causes tuberculosis. At present, nearly all tuberculosis is caused by respiratory infection with M. tuberculosis. Infection may be asymptomatic in some, but in other individuals, it produces pulmonary lesions which lead to severe debilitation or death. Resistance to tuberculosis is provided by cell-mediated immune mechanisms.

Mycobacteria are aerobic, acid-fast, non-spore-forming, non-motile bacili with high lipid contents and slow generation times. M. leprae is the etiologic agent of leprosy and, among the other mycobacteria, the only major pathogen. Bloom, B.R. and T. Godal, Review of Infectious Diseases, 5:765-780 (1983). However, other mycobacterial species are capable of causing disease. Wallace, R.J. et.al., Review of Infectious Diseases, 5:657-679 (1984). M.avium, for example, causes tuberculosis in fowl and in other birds. Members of the M. Avium-intracellularae complex have become important pathogens among individuals with acquired immunodeficiency syndrome (AIDS). Certain groups of

individuals with AIDS have a markedly increased incidence of tuberculosis as well. Pitchenik, A.E. et. al., Annals of Internal Medicine, 101:641-645 (1984).

Diagnostic and immunoprophylatic measures for mycobacterial diseases have changed little in the past half century. Tuberculin, developed by Koch as a cure for tuberculosis in the late 1800s, is an M. tuberculosis filtrate of complex and poorly-defined composition. It is used as a skin test antigen to detect prior exposure to the bacillus. Enrichment of the protein fraction of this material in the 1930's produced the purified protein derivative (PPD) which is still used to diagnose exposure to tuberculosis. Seibert, F.M. et.al., American Review of Tuberculosis, 30(Suppl.):705-778 (1934). Its usefulness is limited, however, by its lack of specificity and its inability to distinguish active disease from prior sensitization by contact with M. tuberculosis or cross-sensitization to other mycobacteria. Young, R.A. and R.W. Davis, Proceedings of the National Academy of Sciences, USA, 80:194-1198 (1983).

Bacille Calmette Guerin (BCG), an avirulent strain of M. bovis, has been used widely as a live vaccine against tuberculosis for over 50 years. Calmette, A., C. et.al., Bulletin of the Academy of

Medicine Paris, 91:787-796 (1924). During that time, numerous studies have shown that BCG has protective efficacy against tuberculosis. These studies are reviewed by F. Luelmo in American Review of Respiratory Diseases, 125(pt. 2):70-72 (1982). However, more recently, a major trial of BCG in India indicated that such a vaccine was not protective against tuberculosis in this setting. World Health Organization WHO Technical Report Series, 651 (1980). Presently available approaches to diagnosing, preventing and treating tuberculosis are limited in their effectiveness and must be improved if a solution is to be found for the important public health problem tuberculosis represents worldwide.

Summary of the Invention

The present invention is based on the isolation of genes encoding immunogenic protein antigens of the tubercle bacillus *Mycobacterium tuberculosis* (*M. tuberculosis*). Genes encoding such protein antigens have been isolated from a recombinant DNA expression library of *M. tuberculosis* DNA. Genes encoding proteins of four additional mycobacteria have also been isolated and restriction maps produced.

In particular, genes encoding five immunodominant protein antigens of the tuberculosis bacillus (i.e., those *M. tuberculosis* proteins of molecular weight 12,000 daltons (12kD), 14kD, 19kD, 65kD and 71kD have been isolated by probing a lambda gt11 expression library of *M. tuberculosis* DNA with

monoclonal antibodies directed against M. tuberculosis-specific antigens.

Recombinant DNA clones producing the specific antigenic determinants recognized by the monoclonal antigens were also isolated in this manner. DNA from such recombinant lambda gt11 clones was mapped with restriction endonucleases; the restriction maps for genes encoding the five immunodominant protein antigens (i.e., genes encoding the 12kD, 14kD, 19kD, 65kD and 71kD proteins) were deduced. The nucleotide sequence of three of the genes have been determined and, in each case, the amino acid sequence of the encoded protein has been deduced.

Brief Description of the Drawings

Figure 1 shows restriction maps of M. tuberculosis DNA. Recombinant DNA clones isolated with monoclonal antibodies directed against the 12kD, 14kD, 19kD, 65kD and 71kD protein antigens were mapped with restriction endonucleases. The insert DNA endpoints are designated left (L) or right (R) in relation to lac Z transcripts which traverse the insert from right to left. Restriction sites are represented as follows: A, Sal I; B, BamHI; E, EcoRI; G, BglII; K, KpnI; P, PvuI; S, SacI; X, XhoI.

Figure 2 shows arrays of antigens from M. tuberculosis recombinant DNA clones probed with rabbit hyperimmune serum. The code of the recombinant DNA clones shown on the numbers filter is: 1, Y3275; 2, Y3274; 3, Y3279; 4, Y3277; 5, Y3247; 6, Y3272; 7, Y3150; 8, Y3254; 9, Y3147; 10, Y3163; 11,

Y3179; 12, Y3191; 13, Y3252; 14, Y3178; 15, Y3180; 16, Y3143; 17, lambda gt11. Clones 1, 5, 6, 7, 9 and 16 are M. tuberculosis recombinants described in the following section. Clones 10, 11, 14 and 15 are M. leprae recombinants expressing epitopes of the 18kD, 28kD, 36kD and 65kD antigens, respectively. Clones 2, 3, 4, 8, 12, 13 are uncharacterized recombinants from the lambda gt11 M. tuberculosis and M. leprae libraries. Clone 17 is a non-recombinant lambda gt11 control.

Figure 3 shows arrays of recombinant mycobacterial antigens probed with monoclonal antibodies to assess the extent of cross-reactivity between recombinant protein antigen of M. tuberculosis and of M. leprae. The array of clones is identical to that shown in Figure 2. Antibody probes and the antigen sizes recognized are: 1, IT-11 (71kD); 2, IT-31 (65kD); 3, IT-16 (19kD); 4, IT-1 (14kD); 5, IT-3 (12kD).

Figure 4 shows restriction maps of DNA encoding four proteins (71kD, 65kD, 19kD and 14kD) of M. tuberculosis and four proteins (71kD, 65kD, 19kD and 14kD) of M. bovis BCG. Restriction sites are represented as follows: A, AatII; B, BamHl; C, BclI; D, DraIII; E=EcoRI; G, BglII; H, HinfI; K, KpnI; P, PstI; S, SalI; V, PvuI and X, XhoI.

Figure 5 is a comparison of restriction maps of the gene encoding the 65kD protein of 6 mycobacteria (M. leprae, M. tuberculosis, M. africanum, M. bovis BCG, M. smegmatis, M. avium). Restriction sites are

as follows: B, BamHl; K, KpnI; N, SacI; P, PvuI; S, SalI; X, XhoI.

Figure 6 is the nucleotide sequence of the region containing the M. tuberculosis 19kD gene. The deduced amino acid sequence of the encoded protein is also represented (protein start position, nucleotide 1110; protein stop position, nucleotide 1586).

Figure 7 is the nucleotide sequence of the region containing the M. tuberculosis 71kD gene and the deduced amino acid sequence of the encoded protein.

Figure 8 is the nucleotide sequence of the region containing the M. tuberculosis 65kD gene. The deduced amino acid sequences of the two long open reading frames are presented in one letter code over (540) or under (517) the appropriate triplets.

Detailed Description of the Invention

The invention described herein is based on the isolation of genes encoding immunogenic protein antigens of the bacillus M. tuberculosis, which is the major etiologic agent of tuberculosis. In particular, it is based on the isolation, using monoclonal antibodies directed against M. tuberculosis-specific antigens, of genes encoding five immunogenic protein antigens of the tuberculosis bacillus; these five antigens are immunodominant. Immunogenic antigens are those which elicit a response from the immune system. Immunodominant protein antigens are immunogenic

antigens against which the immune system directs a significant portion of its response. Genes encoding M. tuberculosis antigens of molecular weight 12,000 daltons (12kD), 14kD, 19kD, 65kD and 71kD were isolated in this manner.

Isolation and characterization of major protein antigens of M. tuberculosis, as described herein, make it possible to develop more effective tools for the prevention, diagnosis, and treatment of tuberculosis. Identification and isolation of genes encoding five immunodominant M. tuberculosis protein antigens, as well as of the five protein antigens, are described below; uses of the genes and encoded products are also described.

M. bovis BCG DNA clones were also isolated for the genes encoding the 71kD, 65kD, 19kD and 14kD proteins. In order to compare M. bovis BCG and M. tuberculosis genes encoding proteins of similar molecular weight, restriction endonuclease maps were determined for DNA segments containing each of the genes. Restriction maps for each of these genes is represented in Figure 4.

In addition, DNA clones were isolated for the genes encoding the 65kD protein from M. africanum, M. smegmatis and M. avium. Restriction endonuclease maps were determined for DNA segments containing each of these genes. The restriction maps for these genes, as well as for the genes encoding the 65kD protein of M. tuberculosis, M. bovis BCG and M. leprae, are represented in Figure 5.

I. Construction of a recombinant expression library of M. tuberculosis DNA

A recombinant DNA expression library of M. tuberculosis DNA was constructed using lambda gt11. The library was constructed with M. tuberculosis genomic DNA fragments in such a way that all protein-coding sequences would be represented and expressed. Young, R.A., B.R. Bloom, C.M. Grosskinsky, J. Ivanyi, D. Thomas and R.W. Davis, Proceedings of the National Academy of Sciences, USA, 82:2583-2587 (1985).

Lambda gt11 is a bacteriophage vector which is capable of driving the expression of foreign insert DNA with E. coli transcription and translation signals. Lambda gt11 expresses the insert DNA as a fusion protein connected to the E. coli Beta-galactosidase polypeptide. This approach ensures that the foreign DNA sequence will be efficiently transcribed and translated in E. coli. This approach is also useful in addressing the problem of the highly unstable nature of most foreign proteins; fusion proteins are often more resistant to proteolytic degradation than is the foreign polypeptide alone. Lambda gt11 and the E. coli strain used (Y1090) have been described previously. Young, R.A. et al., Proceedings of the National Academy of Sciences, USA, 80:1194-1198 (1983); Young, R.A. and R.W. Davis, Science, 222:778-782 (1983). The teachings of these publications are incorporated herein by reference. The library constructed in this manner has a titer of 1x 10^{10} pfu/ml. and

contains approximately 40% recombinants with an average insert size of 4kB.

II. Screening of the lambda gt11 M. tuberculosis library with antibody probes

Murine monoclonal antibodies to protein antigens of M. tuberculosis were used individually to probe the M. tuberculosis recombinant DNA library. This work is described below and with specific reference to the 65kD antigen in the Exemplification. The antibodies used as probes and the sizes of the antigens to which they bind are shown below.

Antibody	M. tuberculosis Antigen
IT-3	12kD
IT-20	14kD
IT-19	19kD
IT-27	19kD
IT-17	23kD
IT-29	23kD
IT-15	38kD
IT-21	38kD
IT-23	38kD
IT-13	65kD
IT-31	65kD
IT-33	65kD
IT-11	71kD

Engers, H.D. et al., Infectious Immunology, 51:718-720 (1986).

All monoclonal antibodies were used at approximately 1:200 to 1:300 dilution in 50mM Tris-HCl pH8/150 mM NaCl/.05% Tween 20.

Screening of the lambda gt11 recombinant DNA library was performed as described by Young et al. in Proceedings of the National Academy of Sciences, USA, 82:2583-2587 (1985), the teachings of which are incorporated herein by reference. One modification was made in the method described by Young and co-workers: 1% bovine serum albumin was used in place of 20% fetal calf serum to decrease background.

Briefly, cloned lambda gt11 recombinants were arrayed on lawns of *E. coli* Y1090. The phage were grown, antigen expression was induced and the antigens were blotted and probed with serum. Detection of signal-producing plaques was performed with a biotinylated secondary antibody system (Vectastain, Vector Laboratories, Burlingame, CA) or with an alkaline phosphatase conjugated secondary antibody system (Protoblot, Promega Biotec, Madison, WI), both used according to manufacturer's instructions. Signal-producing clones were isolated using antibodies directed against protein antigens of molecular weight 12kD, 14kD, 19kD and 65kD and 71kD. In each case, similar numbers of clones were isolated in screens of approximately 10^5 recombinant plaques. DNA clones encoding the 23kD and 38kD antigens could not be detected with these antibodies, possibly because the native epitope is modified or topographically complex, or because the

antigen-antibody interaction is too weak to be recognized by current detection methods.

III. Probing of Arrays of lambda gt11 DNA Clones with Antibody Probes

0.2 ml of a saturated culture of Y1090 was added to 2.5 ml of molten LB soft agar, poured onto 100 mm plates containing 1.5% LB agar and allowed to harden at room temperature for 10 min. 100 ul of phage plate stock containing approximately 10^{11} pfu/ml of the lambda gt11 DNA clones of interest were placed into alternate wells of 96-well tissue culture plates. A multi-pronged transfer device was placed briefly in the wells containing phage and then touched lightly to the surface of the plate onto which the soft agar had been poured. The plates were then incubated at 42°C for approximately 3 hours, at which point clear plaques approximately 5mm in diameter were visible. The plates were then overlayed with nitrocellulose filters saturated with 10mM isopropylthiogalactoside (IPTG) and incubated at 37°C for 3.5 hours. Subsequent processing of filters for detection of antigen was identical to the procedures described for screening of lambda gt11 library with antibody probes.

Immunoscreening of the lambda gt11 library to isolate clones reactive with monoclonal antibodies specific for the 65kD antigen is described in the Exemplification.

IV. Recombinant DNA Manipulation

DNA from recombinant lambda gt11 clones was isolated and mapped with restriction endonucleases by standard techniques. Davis, R.W. et al., Advanced Bacterial Genetics: A Manual for Genetic Engineering, Cold Spring Harbor (1980).

Figure 1 shows the genomic DNA restriction map deduced for each of the genes encoding the five M. tuberculosis antigens and illustrates how each of the cloned DNAs aligns with that map. All clones isolated with monoclonal antibodies directed against any single antigen align with a single genomic DNA segment. This indicates that all clones were isolated because they express the protein of interest rather than an unrelated polypeptide containing a similar or identical epitope. In addition, this result suggests that each antigen is the product of a single gene.

The orientation of each DNA insert in the recombinant clones was determined by restriction analysis. Only among the clones for the 65kD antigen were the inserts found in both possible orientations relative to the direction of lac Z transcription in lambda gt11. This suggests that this protein can be expressed in E. coli from signals independent of those provided by lac Z. Similar results have been obtained for recombinant DNA clones encoding the 65kD antigens of M. bovis and M. leprae. Thole, J.E.R. et al., Infectious Immunology, 50:800-806 (1985); Young, R.A. et al., Nature, 316:450-452 (1985).

The nucleotide sequences of three regions of the M. tuberculosis DNA were determined: 1) the region containing the M. tuberculosis 19kD gene; 2) the region containing the M. tuberculosis 71kD gene; and 3) the region containing the 65kD gene. The three sequences are represented in Figures 6-8. Sequences were determined using standard techniques, which are described in the Exemplification.

V. Filter hybridization of Insert DNA

Arrays of lambda gt11 clones were created as described above and incubated at 42° for 5 hours. The plates were then overlayed with nitrocellulose filters and placed at 4°C for 1 hour. Probe DNA was labelled with ^{32}P by nick translation. Filter hybridization was performed as described by Davis et al. in Advanced Bacterial Genetics: A Manual for Genetic Engineering, Cold Spring Harbor (1980), the teachings of which are incorporated herein by reference. Hybridization conditions were as follows: 50% v/v formamide, 5x SSPE (1x SSPE is 0.18M NaCl, 10mM $Na_{1.5}H_{1.5}PO_4$, 1mM Na_2 EDTA, pH 7.0), 1x Denhardt's solution (0.02% w/v Ficoll, 0.02% w/v polyvinylpyrrolidone, 0.02% w/v bovine serum albumin), 0.3% $NaDodSO_4$ at 42°C for approximately 16 hours, followed by washing in 2x SSPE, 0.2% $NaDodSO_4$ at 45°C.

VI. Recombinant Antigens Recognized by Rabbit Serum

The response of a second animal to an antigen preparation of M. tuberculosis was assessed by

examining the reactivity of rabbit anti-M. tuberculosis hyperimmune sera with recombinant antigens. Cloned lambda gt11 recombinants were arrayed on lawns of E. coli and probed with the rabbit hyperimmune serum. Anti-M. tuberculosis hyperimmune serum, produced by repeated immunization of rabbits with M. tuberculosis H37Rv culture filtrate, was provided by J. Bennedsen (Statens Seruminstitut, Copenhagen, Denmark). These sera were used at 1:100 dilution.

These sera produced positive signals with lambda gt11 clones encoding each of the five M. tuberculosis epitopes which had been isolated with murine monoclonal antibodies (Figure 2). Particularly strong signals were observed with the 65kD and 71kD antigens (Figure 2). These results demonstrate that mice and rabbits can mount an antibody response to the same protein antigens of M. tuberculosis.

Clones for the five M. tuberculosis antigens were detected at similar frequencies in the lambda gt11 recombinant DNA library. Thus, the number and type of antigen-producing clones isolated with polyclonal serum antibodies should reflect the relative titer and deversity of the individual antibodies in this serum.

To determine whether any of the 5 M. tuberculosis antigens are relatively immunodominant in the rabbit humoral immune response to M. tuberculosis, the M. tuberculosis lambda gt11 recombinant DNA library was screened with the rabbit serum. Forty signal-producing clones were isolated, arrayed on

lawns of E. coli Y1090 and probed with monoclonal antibodies directed against each of the 5 recombinant M. tuberculosis protein antigens. Remarkably, 17 of the 40 clones (43%) reacted strongly with at least one of the four anti-65kD monoclonal antibodies tested. An additional six clones (15%) reacted strongly with the anti-17kD monoclonal antibody (IT-11). This indicates that a large proportion of the anti-M. tuberculosis antibody present in the rabbit serum was directed against the 65kD antigen of M. tuberculosis and suggests that it is a dominant antigen for the rabbit humoral immune response. Seventeen of the clones did not react with any of the monoclonal antibodies tested, suggesting that the rabbit sera may identify M. tuberculosis proteins not recognized by the murine antibodies.

VII. Antigenic Relatedness of M. tuberculosis and M. leprae Proteins

There is evidence that M. tuberculosis and M. leprae share immunologically important antigens. To assess this further, an investigation of the exact nature of the immunological relatedness among recombinant protein antigens of M. tuberculosis and M. leprae was conducted.

For each of five M. tuberculosis and four M. leprae protein antigens, a single recombinant DNA clone containing most or all of the gene of interest was used to express antigen in the following manner. The recombinant phage clones were arrayed on a lawn

of _E_. _coli_ Y1090, which was then grown and induced for antigen expression.

Antigen immobilized on nitrocellulose filters was then probed with 26 individual anti-_M_. _tuberculosis_ and _M_. _leprae_ monoclonal antibodies. Figure 3 shows the array of DNA clones used and the results obtained with the anti-M _tuberculosis_ antibodies IT-1, IT-3, IT-11, IT-16, and IT-31, which recognized proteins of 14kD, 12kD, 71kD, 19kD and 65kD respectively. Table 1 details the full results of these cross-screening experiments, showing the reactivity of antigen expressed from individual recombinant DNA clones with each of the individual monoclonal antibodies. Clones were scored as positive only if the signal produced was clearly greater than the background signal produced by the non-recombinant lambda gt11 clone included in each array.

TABLE 1

Reactivity of Monoclonal Antibodies with Recombinant Protein Antigens

		DNA CLONES									
		M. tuberculosis						M. leprae			
ANTIBODIES		12kD Y3275	14kD Y3247	19kD Y3147	65kD Y3150	71kD Y3272	- λgt11	18kD Y3179	28kD Y3163	36kD Y3180	65kD Y3178
M. tuberculosis											
12kD	IT-3	+	-	-	-	-	-	-	-	-	-
14kD	IT-1	-	+	-	-	-	-	-	-	-	-
	IT-4	-	+	-	-	-	-	-	-	-	-
	IT-20	-	+	-	-	-	-	-	-	-	-
19kD	IT-10	-	-	+	-	-	-	-	-	-	-
	IT-12	-	-	+	-	-	-	-	-	-	-
	IT-16	-	-	+	-	-	-	-	-	-	-
	IT-19	-	-	+	-	-	-	-	-	-	-
65kD	IT-13	-	-	-	+	-	-	-	-	-	-
	IT-31	-	-	-	+	-	-	-	-	-	+
	IT-33	-	-	-	+	-	-	-	-	-	+
71kD	IT-11	-	-	-	-	+	-	-	-	-	-
M. leprae											
18kD	L7-15	-	-	-	-	-	-	+	-	-	-
28kD	SA1.D2D	-	-	-	-	-	-	-	+	-	-
	SA1.BIIH	-	-	-	-	-	-	-	-	-	-
36kD	F47-9-1	-	-	-	-	-	-	-	-	+	-
	ML04-A	-	-	-	-	-	-	-	-	-	-
65kD	CI.I	-	-	-	+	-	-	-	-	-	+
	IIH9	-	-	-	+	-	-	-	-	-	+
	IIIE9	-	-	-	-	-	-	-	-	-	+
	IIC8	-	-	-	+	-	-	-	-	-	+
	IIIC8	-	-	-	-	-	-	-	-	-	+
	T2.3	-	-	-	+	-	-	-	-	-	+
	Y1-2	-	-	-	+	-	-	-	-	-	+
	SA2.D7C	-	-	-	+	-	-	-	-	-	+
	ML30A	+	+	+	+	+	+	+	+	+	+

Several conclusions can be drawn from the results shown in Table 1. Among the 11 monoclonal antibodies that recognize a 65kD antigen, 7 react with the 65kD protein from both mycobacteria (IT-31, C1.1, IIH9 (identical to IT-33), IIC8, T2.3, Y1-2, SA2.D7C), one antibody reacts only with the M. tuberculosis 65kD protein (IT-13), and two antibodies react only with the M. leprae 65kD protein (IIIE9 and IIIC8). One antibody, ML30A, cross-reacts with an antigen in E. coli and could not specifically identify antigen-producing clones. These results indicate that the 65kD protein antigens of M. tuberculosis and M. leprae are homologues and share a number of epitopes. In addition to these shared epitopes, however, both 65kD antigens have epitopes that are specific for one species relative to the other.

No cross-reactivity was observed between other antigens of these two mycobacterial species. Because monoclonal antibodies recognize a single epitope and because only one or a few antibodies were available for each antigen, it is not clear whether the 65kD proteins are the only homologous protein antigens of M. tuberculosis and M. leprae. Among the antigens for which lambda gt11 clones have been isolated, the 18kD antigen of M. leprae and the 19kD antigen of M. tuberculosis are of similar size. To determine whether these two antigens are related, the homology of the DNA sequences that encode these antigens was examined. At conditions of moderate stringency, no hybridization was observed between

the insert DNA and Y3147 (an M. tuberculosis 19kD clone) and Y3179 (an M. leprae 18kD clone). This indicates no significant homology between the DNA sequences of the insert DNAs of these two clones. This result suggests that the M. tuberculosis 19kD and the M. leprae 18kD proteins are unlikely to be homologous.

As a result of the work described, recombinant DNA clones encoding five major protein antigens of M. tuberculosis were isolated through the use of an extensive collection of well-characterized murine monoclonal antibodies. These five proteins were also found to be major antigens in the rabbit humoral immune response to M. tuberculosis. One of these antigens, the 65kD protein, is shared with another other mycobacterial pathogen M. leprae.

Several lines of evidence indicate that the 65kD antigen is among the most immunodominant of the protein antigens of M. tuberculosis. Eleven of the 25 different M. tuberculosis and M. leprae monoclonal antibodies examined in this study recognized the 65kD recombinant antigen from one or both mycobacteria. In addition, almost half of the recombinant DNA clones isolated with rabbit polyclonal anti-M. tuberculosis sera express the 65kD antigen, reflecting the predominance of antibody to this antigen in these sera.

Considerable evidence indicates that the 65kD antigen plays an important role in the human response to tuberculosis. Antibodies directed against this protein can be detected in the serum of

patients with tuberculosis. The 65kD antigen is present in purified protein derivatives (PPD's) of M. tuberculosis, M. bovis, and other mycobacteria. Thole, J.E.R. et al., Infection Immunity, 50:800-806 (1985). Finally, helper T cell clones reactive with recombinant 65kD antigen have been isolated from patients with tuberculosis, indicating that this antigen is involved in the cell-mediated as well as the humoral immune response to tuberculosis.

Among the major antigens of the leprosy bacillus, the 65kD antigen appears to elicit antibody and T cell responses similar to those observed for the M. tuberculosis antigen. Both serum antibodies and T cells directed against the 65kD M. leprae antigen have been observed in patients with leprosy. Britton, W.J. et al., Journal of Immunology, 135:4171-4177 (1985); Mustafa, A.S. et al., Nature, 319:63-66 (1986). In addition, T cell clones from leprosy patients have been found to respond to recombinant 65kD protein of M. bovis, as well as to PPD's from both M. bovis BCG and M. leprae. Emmrich, F. et al., Journal of Experimental Medicine, 163:1024-1029 (1986); Shankar, P. et al., Journal of Immunology, 136:4255-4263 (1986). It is interesting to note that in vaccine trials in Asia and Africa, BCG provided significant protection against leprosy, ranging from 20% to 80%. Fine, P., Tubercle, 65:137-153 (1984). An intriguing possibility is that the M. bovis BCG 65kD antigen is involved in engendering the immune protection

provided by this vaccine against *M. leprae*, as well as against *M. tuberculosis*.

In addition to the 65kD antigen, there is evidence that the 19kD and 71kD antigens of *M. tuberculosis* may be particularly important in the immune response to this bacillus. Helper T cell clones from tuberculosis patients have been isolated which respond to the recombinant 19kD protein. The 71kD antigen is recognized by the humoral immune system of both mice and rabbits, and antibody to this antigen has been shown to be a prominent component of hyperimmune anti-*M. tuberculosis* rabbit sera.

VIII. Isolation of DNA Clones for Genes Encoding Proteins of Additional Mycobacteria

Using the procedures described above for isolation of genes encoding *M. tuberculosis* proteins, genes encoding proteins of additional mycobacteria were isolated. DNA clones containing genes encoding the following proteins were isolated:

Mycobacterium	Protein	Clone
M. bovis BCG	71kD	PL1-101
	65kD	PL1-105
	19kD	PL1-501
	14kD	PL1-502
M. smegmatis	65kD	PL1-206
M. avium	65kD	PL1-401
M. africanum	65kD	PL1-301

For purposes of comparison, genes encoding the following proteins were isolated for M. tuberculosis and M. leprae:

Mycobacterium	Protein	Clone
M. tuberculosis	71kD	Y3272
	65kD	Y3150
	19kD	Y3147
	14kD	Y3248
M. leprae	65kD	

The following strains were used for this purpose:

Species	Isolate
M. leprae	Armadillo isolate (WHO)
M. tuberculosis	Erdmann strain
M. africanum	African clinical isolate
M. bovis BCG	Danish vaccine strain
M. smegmatis	MC^2-6
M. avium	AIDS patient isolate

DNA from recombinant lambda gt11 clones was isolated, as described above, and mapped with restriction endonucleases, using standard techniques. Davis, R.W. et al., Advanced Bacterial Genetics: A Manual for Genetic Engineering, Cold Spring Harbor (1980).

Figure 4 presents a comparison of the restriction maps for four genes of M. tuberculosis with the restriction maps for four genes of M. bovis BCG which encode proteins of the same molecular weight. As is evident from the figure, in each case, the

restriction sites on the two genes (e.g., those on the M. tuberculosis gene and those on the M. bovis gene which encodes a protein of the same molecular weight) are essentially identical. This indicates that the sequence of the genes of the two mycobacteria (at least those encoding these four proteins) are very similar and, therefore, the proteins they encode are also very similar.

Figure 5 presents a comparison of the restriction map for the gene encoding the 65kD protein for the six mycobacteria. As is evident, the restriction maps for the genes encoding the 65kD protein of M. tuberculosis, M. africanum, M. bovis BCG, M. smegmatis and M. avium are essentially identical. The fact that there is no detectable difference among these mycobacteria at the level of the restriction map is an indication that, at least at this level, the encoded proteins are the same.

As is also evident, the map of the M. leprae 65kD gene has several identical restriction sties in common with those of the other mycobacteria; it also has two sites not found in the other genes and lacks three sites present in the others. This indicates that, at the level of the restriction map, there are similarities in the DNA (and the encoded protein). In addition, however, there are differences apparent at this level.

IX. Diagnostic, Therapeutic and Preventive Applications

The isolation of genes encoding major protein antigens of M. tuberculosis makes it possible to

address problems which presently exist in diagnosing treating and preventing tuberculosis. Isolation of genes encoding proteins of other mycobacteria, such as M. bovis BCG, M. africanum, M. smegmatis and M. avium makes it possible to address similar problems in diseases which they cause.

The nucleotide sequence of three of the five genes has been determined. The sequence of the remaining genes can be determined using well-known methods, such as that of Sanger et al. Sanger, F. et.al., Proceedings of the National Academy of Sciences, USA, 74:5463-5467 (1977). The amino acid sequence of each of the immunodominant proteins has been deduced from the nucleotide sequence of the three genes and can be done for the others.

Identification and characterization of the genes for major tuberculosis protein antigens and of the proteins themselves make it possible to develop improved reagents for diagnosis and immunoprophylaxis of tuberculosis. Proteins antigens encoded by an entire gene, or amino acid sequences (e.g., peptides, protein fragments) which make up the antigenic determinant of a M. tuberculosis antigen (i.e., M. tuberculosis-specific antigenic determinants) may be used in serodiagnostic tests and skin tests. Such antigens would be highly specific to the tuberculosis bacillus and the tests in which they are used would also be highly specific. Highly specific serological tests would be of great value in screening populations for

individuals producing antibodies to M. tuberculosis-specific antigenic determinants; in monitoring the development of active disease in individuals and in assessing the efficacy of treatment. As a result, early diagnosis of tuberculosis will be feasible, thus making it possible to institute treatment at an early stage of the disease and, in turn, to reduce the likelihood it will be transmitted.

As a result of the work described, it is also possible to determine which segment(s) of the M. tuberculosis antigen is recognized by M. tuberculosis-specific T cells. A mixture of peptides recognized by helper T cells can serve as a specific skin test antigen useful in assessing immunological status (delayed hypersensitivity) of infected individuals and those with whom they come in contact. This specific skin test antigen would be useful in evaluating rapidly the immunological efficacy of anti-tuberculosis vaccines.

It is reasonable to expect that the products encoded by M. tuberculosis genes, particularly those shown to be recognized by helper T cells, are themselves immunogenic and thus useful components of vaccines against tuberculosis. These products include proteins and portions of such proteins (e.g., polypeptides and peptides). For example, one approach to vaccine development is the introduction of genes encoding products (e.g., polypeptides) which provide immunological protection into viruses such as vaccinia virus, or bacteria, such as cultivatable mycobacteria, thus producing a vaccine

capable of engendering long-lasting and very specific immunity. The genes encoding five immunodominant protein antigens of the tuberculosis bacillus, described herein, are useful for that purpose; genes encoding the 65kD, 19kD and 71kD antigens, or a portion thereof, are particularly valuable in vaccine construction.

Because of the similarities in the DNA encoding similarly-sized proteins and, thus, of the encodied proteins themselves, it is possible that, for example, a vaccine effective against two or more of the mycobacteria can be produced.

EXEMPLIFICATION

Isolation and Analysis of Recombinants Expressing the 65kD M. tuberculosis Antigen

The recombinant DNA library of M. tuberculosis genomic DNA fragments in the lambda gt11 vector was constructed as described above. Recombinant phage lambda RY3143 and lambda RY3146 were used. Young, R.A. et al., Proceedings of the National Academy of Sciences, USA, 82:2583-2587 (1985). Subclones of the mycobacterial DNA inserts in these recombinant phage were constructed in pUC19 or M13mp9 vectors using standard recombinant DNA techniques. Messing, J. and J. Viera, Gene, 19:269-276 (1982). Maniatis, T. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982).

Monoclonal antibodies specific for the 65kD antigen were obtained from the Immunology of Tuberculosis Scientific Working Group under a grant from the WHO/World Bank/UNDP Special Program for Vaccine Development. These antibodies included IT-13 (WTB-78), IT-31 (SA2D5H4), and IT-33 (MLIIH9). Coates, A.R.M. et al., Lancet, 2:167-169 (1981). Gillis, T.P. and T.M. Buchanon, Immunology, 37:172-178 (1982). Anti-B-galactosidase antibodies were purchased from CooperBiomedical. Polyclonal rabbit antisera directed against a sonicate of M. tuberculosis strain H37Rv were elicited as described by Minden and co-workers. Minden, P. et al., Infect. Immun., 46:519-525 (1984). Results are shown in Table 2.

TABLE 2: PATTERNS OF ANTIBODY REACTIVITIES[a]

Number of Clones	Reactivity with Antibodies		
	IT-13	IT-31	IT-33
27	+	+	+
1	+	+	+
2	+	-	+
3	-	+	+
1	+	-	-
2	-	+	-
2	-	-	+

[a]: Recombinant clones expressing antigens reactive with the 65kD antigen specific monoclonal antibodies IT-13, IT-31, and IT-33 were isolated as described above. For the initial screen, a pool of the three antibodies was used; it contained a 1:1000 dilution of each antibody to screen a total of about 8×10^5 recombinant phage from the lambda gt11-*M. tuberculosis* library. To determine which monoclonal antibody reacted with which of the 38 plaque-purified recombinants, about 100 pfu of each recombinant phage were inoculated in small spots on a lawn of Y1090. The phage were allowed to grow and induced to synthesize the foreign proteins as described previously. The filters were then reacted with a 1:1000 dilution of one of the monoclonal hybridoma antibodies as described above.

The lambda gt11-M. tuberculosis library was screened with the monoclonal antibodies specific for the 65kD antigen and clones reactive with them were isolated essentially as described by Young et al. Young, R.A. et al., Proceedings of the National Academy of Sciences, USA, 82:2583-2587 (1985). Briefly, for each 150mm LB plate, 0.6ml of a fresh overnight culture of Y1090 was infected with 1-2 $x10^5$ plaque forming units of the library. After 3.5-4 hours of growth at 42°C, the plaques were overlaid with a dry nitrocellulose filter which had been saturated with 10mM isopropyl-B-D-thiogalacto-pyranoside (IPTG). The plates were incubated an additional 3.5-4 hours at 37°C and then removed to room temperature and the position of the filters marked. The filters were washed briefly in TBST (50 mM Tris-HCl, pH 8, 150mM NaCl, 0.05% Tween 20) and then incubated in TBST + 20% fetal calf serum. After 30 minutes at room temperature, the filters were transferred to TBST plus antibody. For the initial screen, the antibody mix contained a 1:1000 dilution of IT-13, IT-31, and IT-33. The filters were incubated with the antibody solution overnight at 4°C with gentle agitation, washed in TBST and reacted with biotinylated goat anti-mouse immuno-globulin, the Vectastain ABC reagent, and developer as described by the manufacturer (Vector Laboratories). After the color had developed the filters were washed with several changes of water and air dried. Phage corresponding to positive signals were twice plaque purified. To determine

which monoclonal antibodies reacted with which of the recombinant phage, about 100 pfu of a purified phage stock were inoculated in a small spot on a lawn of Y1090 bacteria on an LB plate. The phage were allowed to grow and induced to synthesize the foreign proteins as described above. The filters were then reacted with a 1:1000 dilution of one of the monoclonal antibodies. The subsequent steps were the same as for the initial screen.

Western blot assays were carried out as follows: Cells containing phage or plasmids in which the expression of the foreign sequences was under the control of the E. coli lac gene regulatory sequences were induced to synthesize the foreign proteins by incubating the cells in the presence of 2.5mM IPTG for 2 hours. Crude lysates of cells expressing lambda gt11 recombinants were made as described in Huynh et al. Huynh, T.V. et al., In: DNA Cloning Techniques: A Practical Approach, (D. Glover, ed.) IRL Press, Oxford, Vol. 1, pp. 49-78 (1985). Crude lysates of cells expressing plasmid encoded proteins were made by harvesting cells from overnight cultures and resuspending the cells in 10 mM Tris pH7.5/10 mM EDTA containing 100 ug lysozyme/ml. After 10 minutes at room temperature, SDS was added to a final concentration of 0.5%. A protease inhibitor (Trasylol, Boehringer Mannheim) was added to all crude lysates at a final concentration of 0.3%. The crude protein preparations were electrophoresed on 10% polyacrylamide-SDS Laemmli gels and the separate proteins electrophor-

etically transferred to nitrocellulose. Laemmli, U.K., Nature, 227:680-685 (1970). Towbin, H. et al., Proceedings of the National Academy of Sciences, USA, 76:4350-4354 (1979). The immobilized proteins were reacted with a 1:1000 dilution of monoclonal antibody IT-13 in TBST overnight at 4°C. The nitrocellulose filters were then washed, reacted with peroxidase-conjugated goat anti-mouse immunoglobulin, and developed as described by Niman and co-workers. Niman, H.L. et al., Proceedings of the National Academy of Sciences, USA, 80:4949-4953 (1983).

The sequences of 5'-end-labeled restriction fragments of the mycobacterial DNA were determined by a modification of the partial chemical degradation technique of Maxam and Gilbert. Brow, M.A.D. et al., Mol. Biol. Evol., 2:1-12 (1985). Maxam, A.M. and W. Gilbert, Proceedings of the National Academy of Sciences, USA, 74:560-564 (1976). For the M13/dideoxy sequencing studies, Sau3AI fragments from the mycobacterial DNA inserts were subcloned into the BamHI site of M13mp9. Phage DNA was isolated from the M13 recombinants and subjected to the dideoxy chain termination sequencing reactions. Biggin, M.D. et al., Proceedings of the National Academy of Sciences, USA, 80:3963-3965 (1983). Sanger, F. et al., Journal of Molecular Biology, 143:161-178 (1980). The products of the sequencing reactions were electrophoresed on 6% acrylamide/7M urea/0.5-2.5 x TBE gradient sequencing gels. The gels were dried

under vacuum and exposed to Kodak XRP-1 film. The nucleotide sequences were determined independently for both strands of the mycobacterial DNA.

Computer-aided analyses of the nucleic acid sequences and deduced protein sequences were performed using the Databases and programs provided by the Nucleic Acid and Protein Identification Resources of the National Institutes of Health as well as the programs of Chou and Fasman and Hopp and Woods. Chou, P.Y. and G.D. Fasman, Adv. Enzym., 47:45-148 (1978). Hopp, T.P. and K.P. Woods, Proceedings of the National Academy of Sciences, USA, 78:3824-3828 (1981). The nucleotide sequence of the region containing the M. tuberculosis 65kD gene and the deduced amino acid sequence of the two long open reading frames are represented in Figure 8.

B-galactosidase assays were also carried out. Cells were grown in LB broth or LB broth plus 2.5mM IPTG to an OD_{600} of about 0.3. Crude lysates were made and b-galactosidase activity assayed as described by Miller. Miller, J.H., Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1972).

Equivalents

Those skilled in the art will recognize or be able to ascertain, using no more than routine experimentation, many equivalents to the specific materials and components described herein. Such equivalents are intended to be encompassed in the scope of the following claims.

CLAIMS

1. Isolated DNA encoding an immunogenic protein antigen of Mycobacterium tuberculosis.

2. DNA of Claim 1 selected from the group consisting of DNA encoding Mycobacterium tuberculosis protein antigens of molecular weight 71kD, 65kD, 19kD, 14kD and 12kD.

3. Isolated DNA encoding an immunodominant protein antigen of Mycobacterium tuberculosis, the protein antigen having a molecular weight of approximately 65kD and recognized by a monoclonal antibody selected from the group consisting of: IT-31; C1.1; IIH9; IIC8; T2.3; Y1-2; SA2.D7C and IT-13.

4. Isolated DNA encoding an immunodominant protein antigen of Mycobacterium tuberculosis, the protein antigen having a molecular weight of approximately 19kD and recognized by a monoclonal antibody selected from the group consisting of: IT-10; IT-12; IT-16; and IT-19.

5. Isolated DNA encoding an immunodominant protein antigen of Mycobacterium tuberculosis, the protein antigen having a molecular weight of approximately 71kD and recognized by the monoclonal antibody IT-11.

6. Isolated DNA encoding an antigenic determinant of <u>Mycobacterium tuberculosis</u> protein.

7. DNA of Claim 6 which encodes an antigenic determinant selected from the group consisting of antigenic determinants of <u>Mycobacterium tuberculosis</u> proteins of molecular weight 71kD, 65kD, 19kD, 14kD and 12kD.

8. Isolated DNA encoding an amino acid sequence of an antigenic determinant of <u>Mycobacterium tuberculosis</u> protein, said protein having a molecular weight of approximately 65kD.

9. Isolated <u>Mycobacterium tuberculosis</u> DNA encoding an immunodominant protein antigen having a molecular weight of approximately 65kD, said DNA selected from the group consisting of:
   a. the DNA insert of clone Y3141;
   b. the DNA insert of clone Y3143;
   c. the DNA insert of clone Y3150;
   d. the DNA insert of clone Y3253; and
   e. the DNA insert of clone Y3262.

10. A protein antigen encoded by DNA of Claim 9.

11. A protein antigen of Claim 10, wherein the protein antigen is recognized by a monoclonal antibody selected from the group consisting of IT-31; C1.1; IIH9; IIC8; T2.3; Y1-2; SA2.D7C and IT-13.

12. Isolated DNA having a nucleotide sequence selected from the group consisting of: a) the nucleotide sequence represented in Figure 6, or a portion thereof; b) the nucleotide sequence represented in Figure 7, or a portion thereof; and c) the nucleotide sequence represented in Figure 8, or a portion thereof.

13. A protein or a peptide selected from the group consisting of: a) proteins or peptides encoded by the nucleotide sequence represented in Figure 6, or a portion thereof; b) proteins or peptides encoded by the nucleotide sequence represented in Figure 7, or a portion thereof; and c) proteins or peptides encoded by the nucleotide sequence represented in Figure 8, or a portion thereof.

14. A peptide having the amino acid sequence of an antigenic determinant of Mycobacterium tuberculosis protein, said antigenic determinant being unique to Mycobacterium tuberculosis protein.

15. A peptide encoded by isolated Mycobacterium tuberculosis DNA, said peptide recognized by helper T cells.

16. A peptide encoded by the Mycobacterium tuberculosis DNA insert of clone Y3150 or a portion of said DNA insert.

17. Isolated DNA encoding a protein of _Mycobacterium africanum_ the protein having a molecular weight of 65kD.

18. Isolated DNA encoding a protein of _Mycobacterium avium_, the protein having a molecular weight of 65kD.

19. A vaccine comprising DNA encoding _Mycobacterium tuberculosis_ protein in a recombinant vaccine vector capable of expressing said DNA.

20. A vaccine of Claim 19 in which the recombinant vaccine vector is vaccinia virus or cultivatable mycobacteria.

21. A vaccine of Claim 20 in which the DNA encodes the 65kD _Mycobacterium tuberculosis_ protein recognized by the monoclonal antibody IT-13, or a portion of said protein.

22. A vaccine comprising DNA encoding an antigenic determinant unique to _Mycobacterium tuberculosis_ cultivatable mycobacteria capable of expressing said DNA.

23. A method of detecting antibody against _Mycobacterium tuberculosis_ in a biological fluid, comprising the steps of:

a) incubating an immunoadsorbent comprising a solid phase to which is attached

immunodeterminant Mycobacterium tuberculosis protein with a sample of the biological fluid to be tested, under conditions which allow the anti-Mycobacterium tuberculosis antibody in the sample to bind to the immunoadsorbent;

b) separating the immunoadsorbent from the sample; and

c) determining if antibody is bound to the immunoadsorbent, as an indication of anti-Mycobacterium tuberculosis in the sample.

24. A method of Claim 23 in which the Mycobacterium tuberculosis protein attached to the solid phase has a molecular weight of approximately 65kD.

25. A method of detecting antibody against Mycobacterium tuberculosis in a biological fluid, comprising the steps of:

a) incubating an immunoadsorbent comprising a solid phase to which is attached a peptide having the amino acid sequence of an antigenic determinant of Mycobacterium tuberculosis protein with a sample of the biological fluid to be tested, under conditions which allow antibody against Mycobacterium tuberculosis to bind to the immunoadsorbent;

b) separating the immunoadsorbent; and

c) determining if antibody is bound to the immunoadsorbent, as an indication of the

presence of the antibody against *Mycobacterium tuberculosis* in the sample.

26. A method of Claim 25 in which the peptide has the amino acid sequence of an antigenic determinant which is unique to *Mycobacterium tuberculosis* protein.

27. A kit useful in detecting antibody against *Mycobacterium tuberculosis* in a biological fluid, comprising a collection of reagents for immunoassay of said antibody, said collection of reagents a solid phase to which is attached immunodeterminant *Mycobacterium tuberculosis* protein or a peptide having the amino acid sequence of an antigenic determinant of *Mycobacterium tuberculosis*.

FIGURE 1

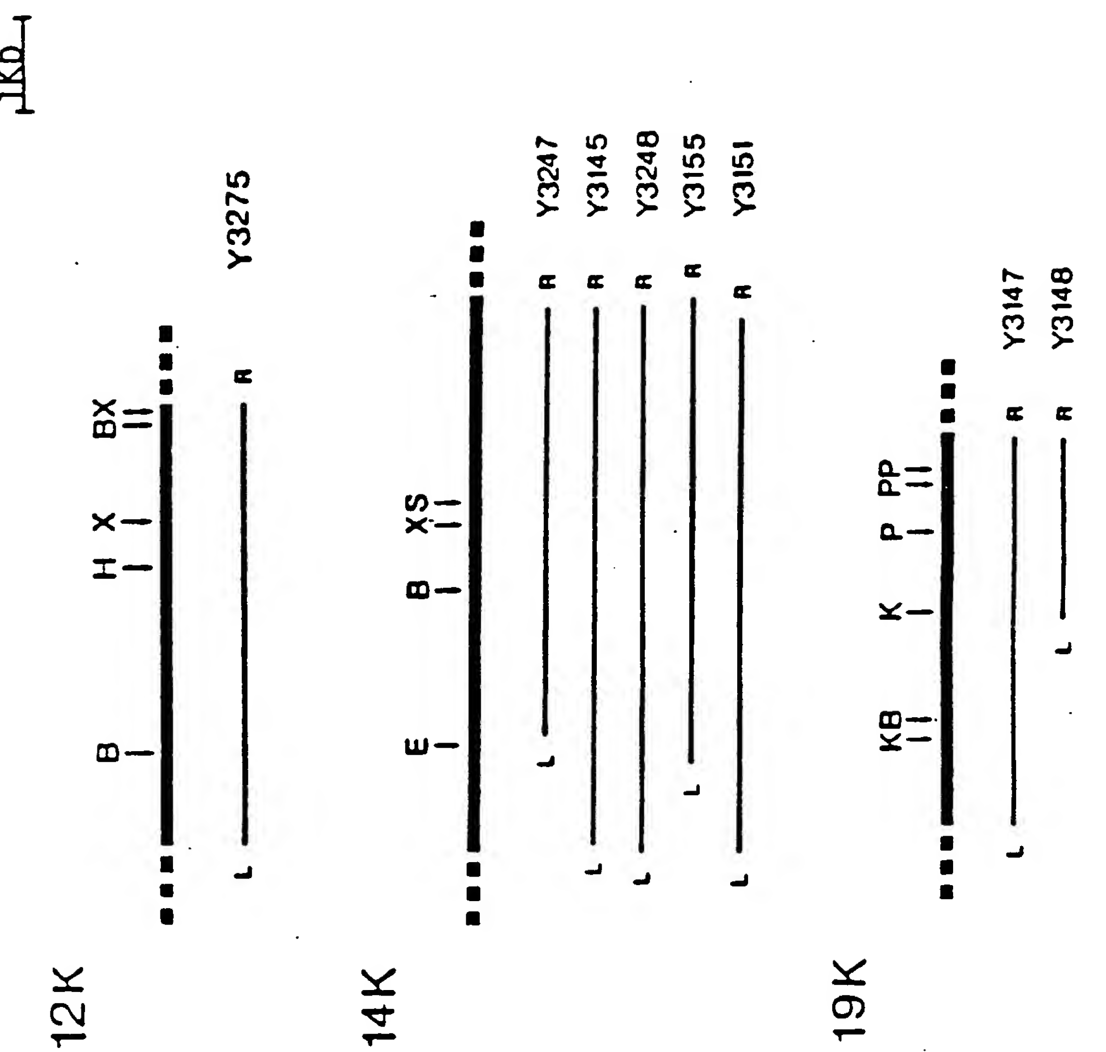

1Kb
12K
B
H X
BX
Y3275
14K
E
B
XS
Y3247
Y3145
Y3248
Y3155
Y3151
19K
KB
K
P
PP
Y3147
Y3148

FIGURE 1 (Cont'd)

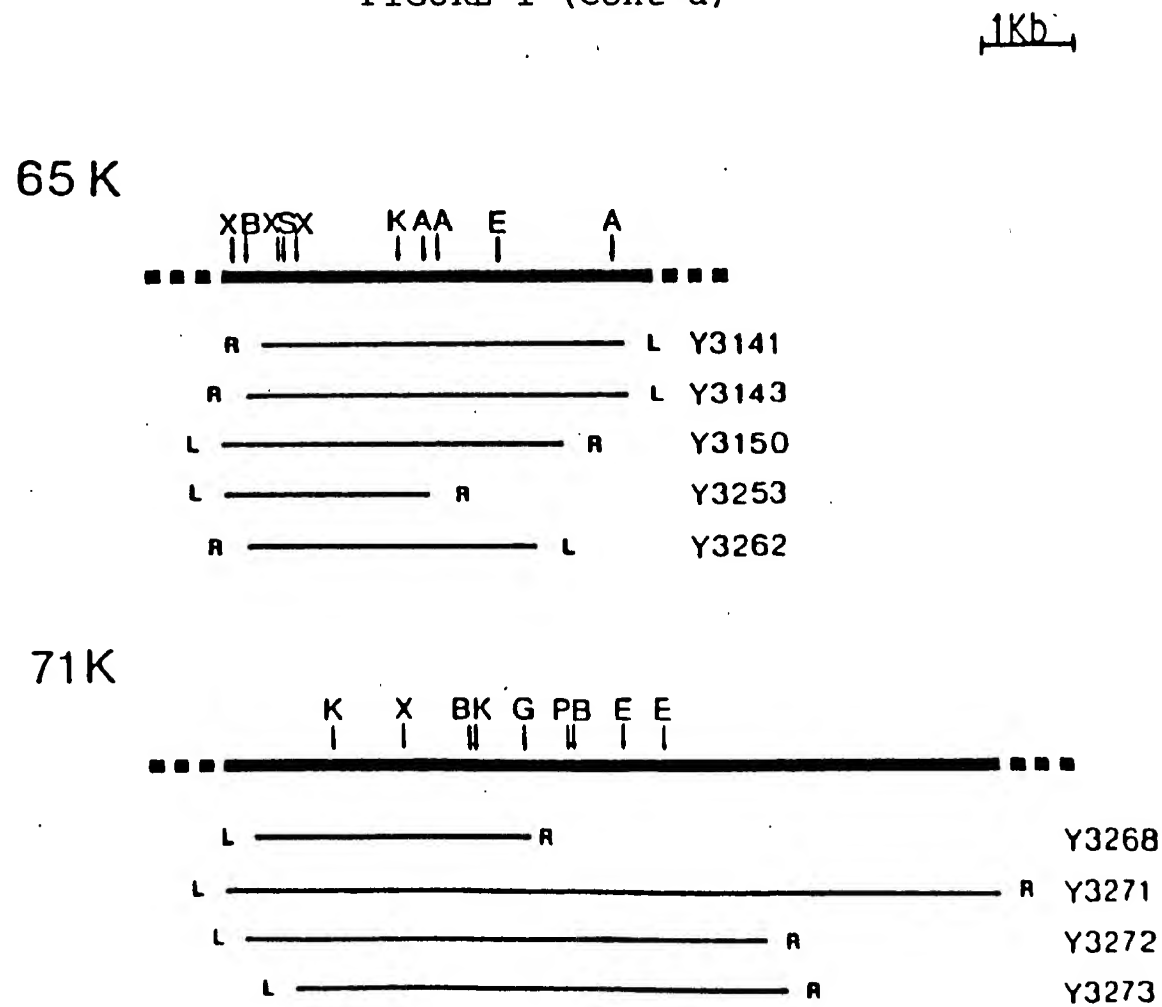


FIG.2

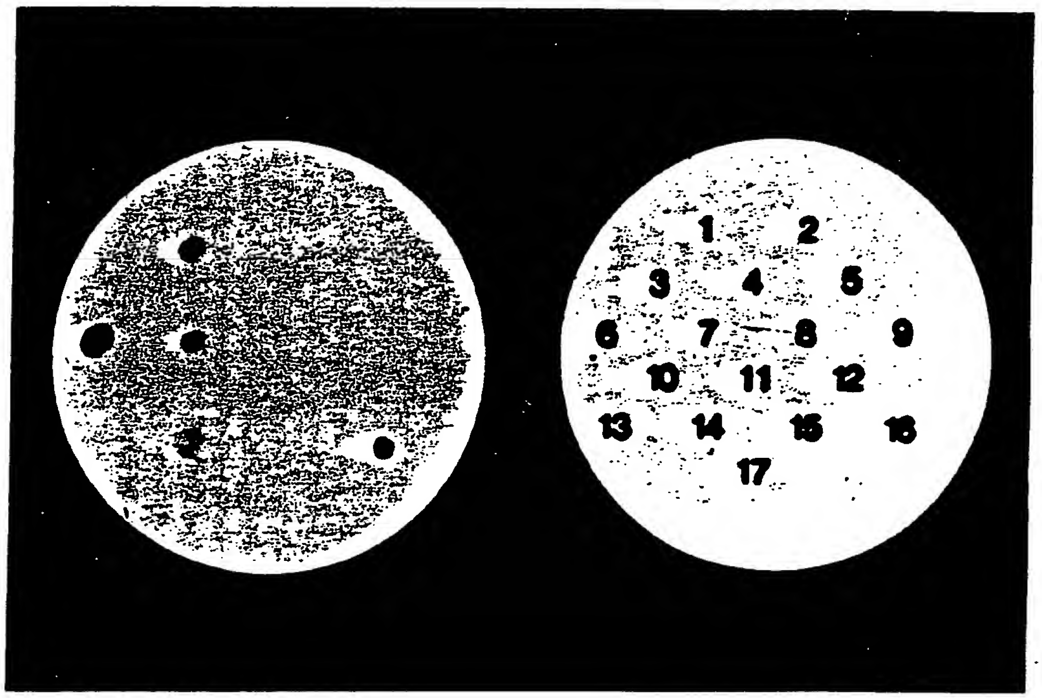


FIG.3

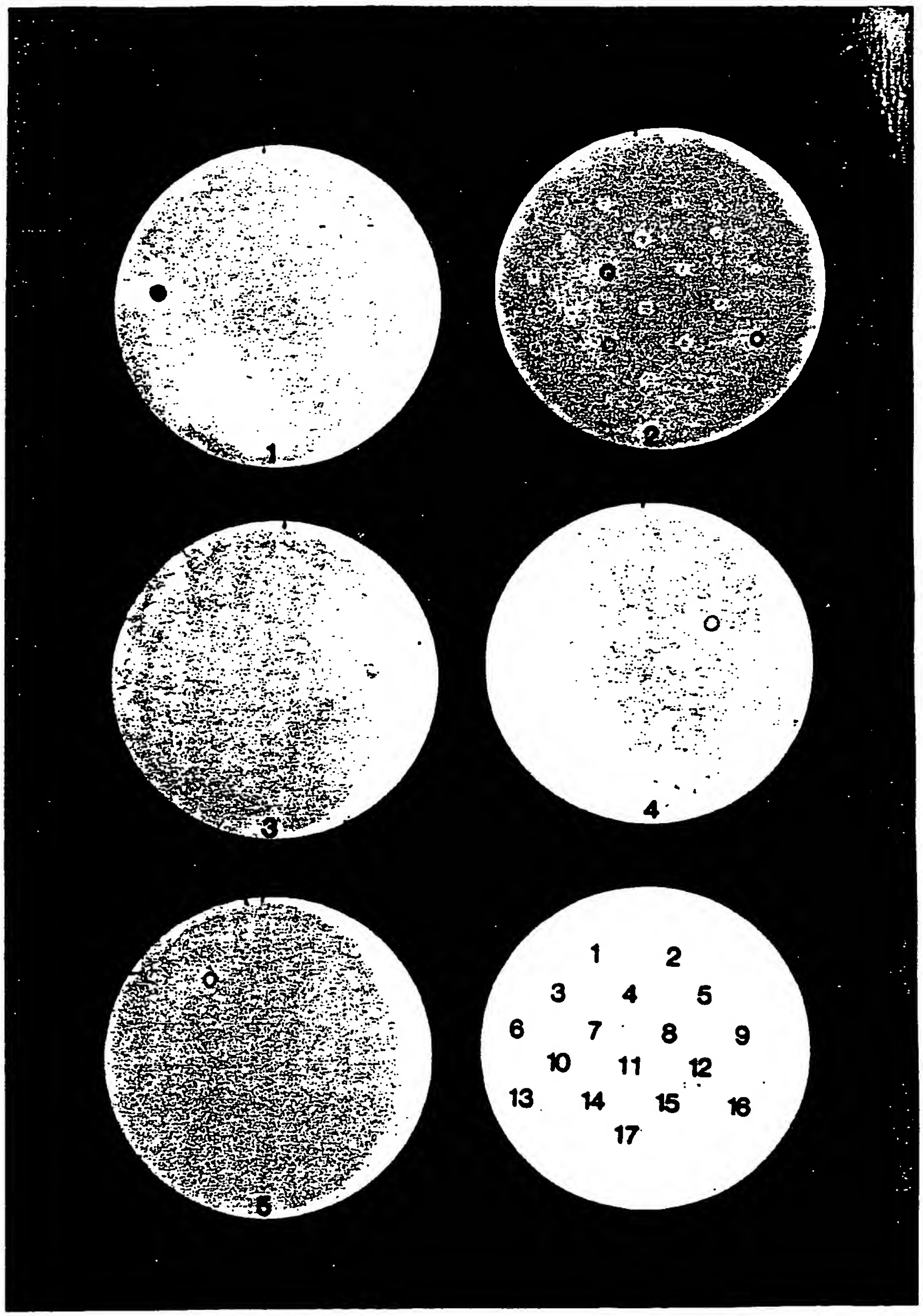


FIGURE 4

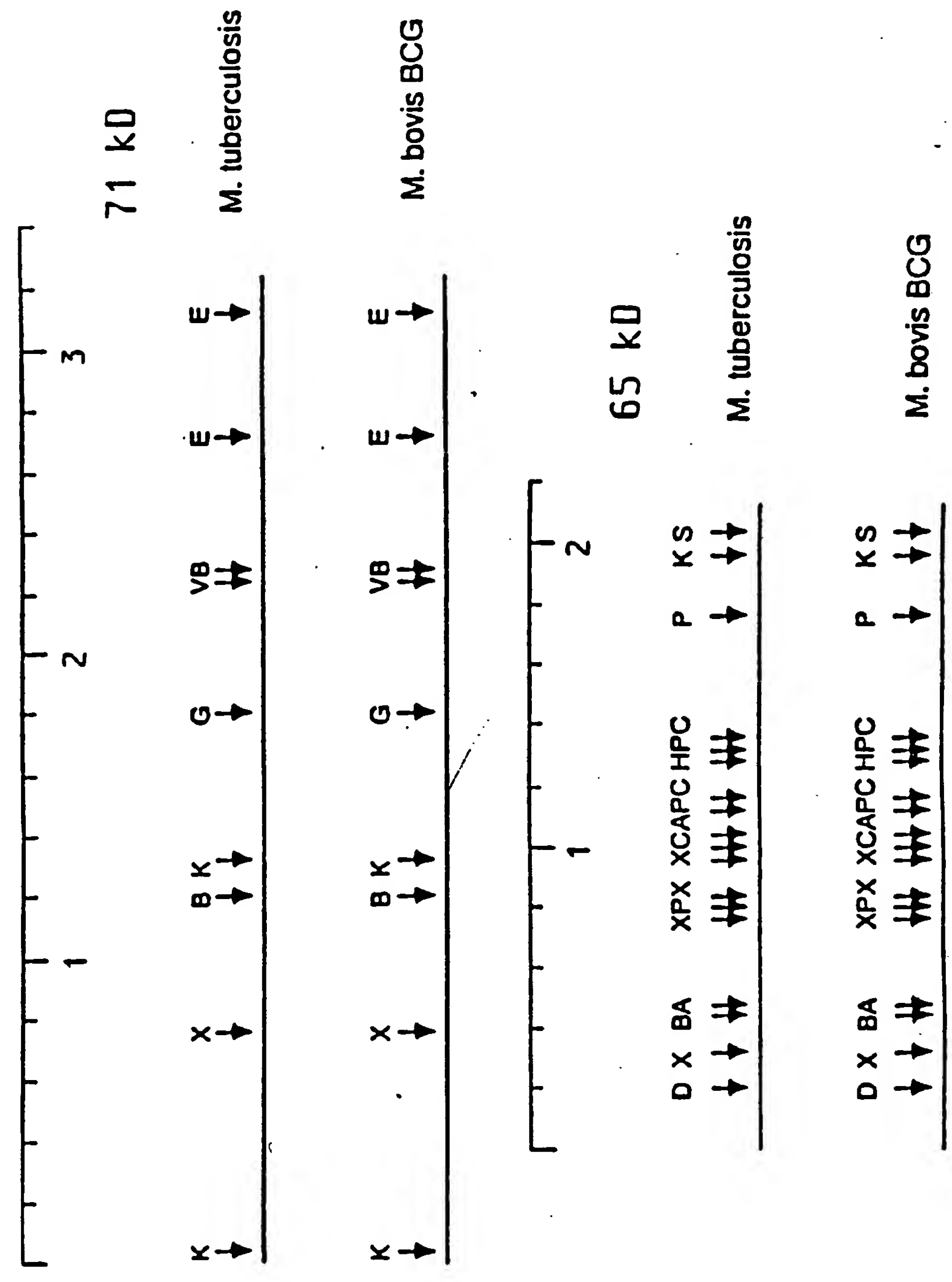
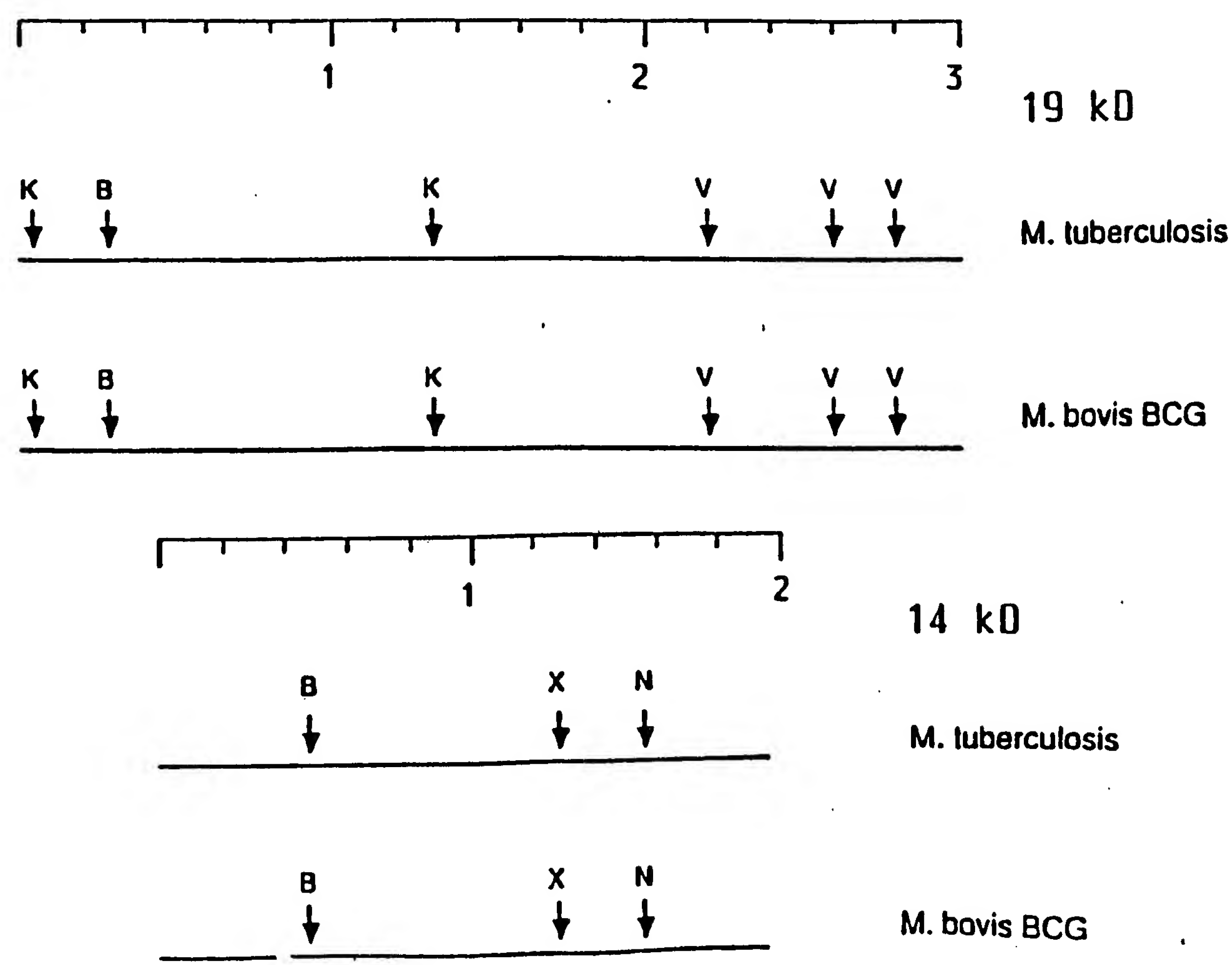

71 kD
M. tuberculosis
M. bovis BCG
K X B K G VB E E
1 2 3
65 kD
M. tuberculosis
M. bovis BCG
D X BA XPX XCAPC HPC P K S
1 2

FIGURE 4 (Cont'd)



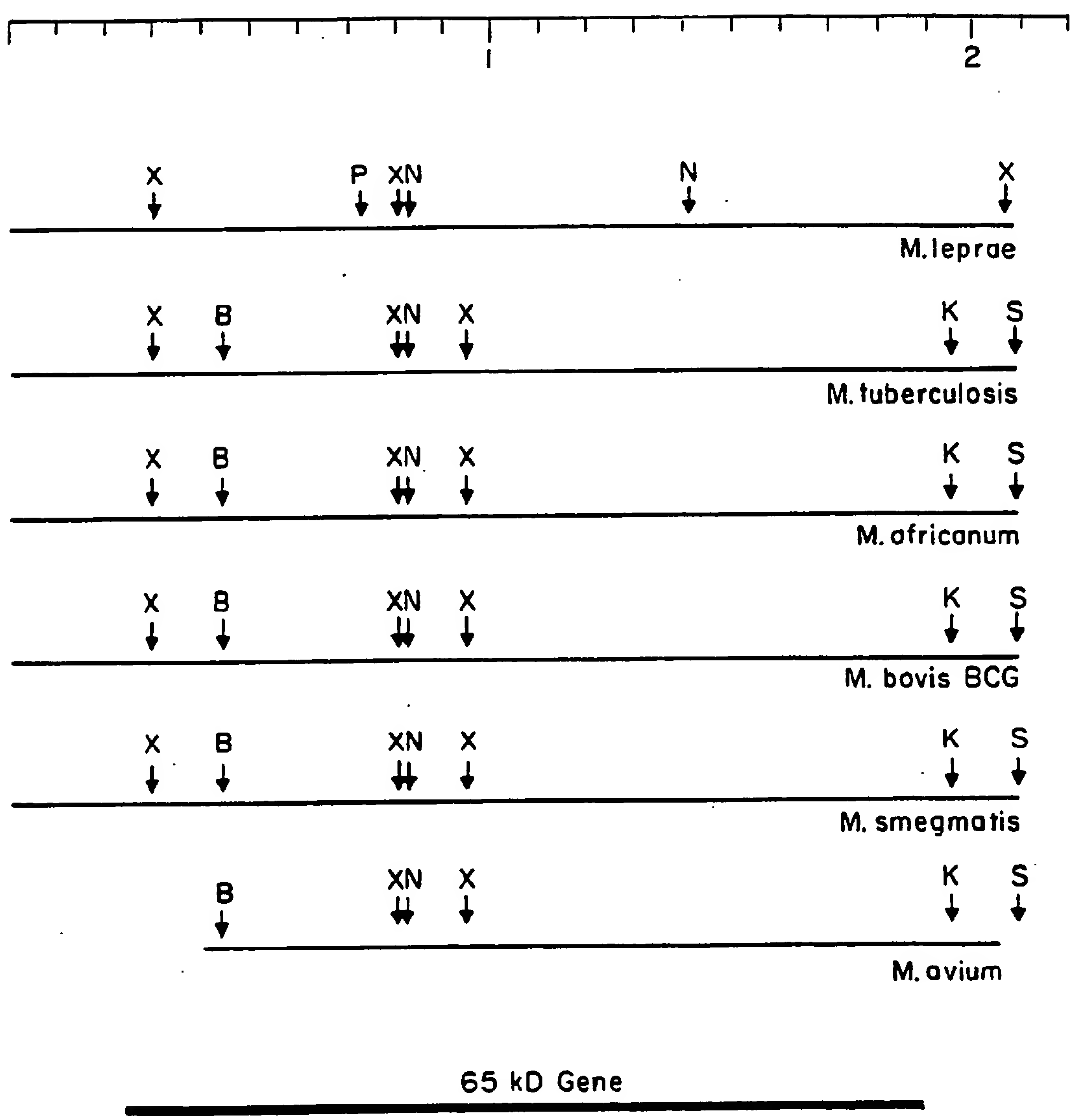

1
2
X
P
XN
N
X
M. leprae
X
B
XN
X
K
S
M. tuberculosis
X
B
XN
X
K
S
M. africanum
X
B
XN
X
K
S
M. bovis BCG
X
B
XN
X
K
S
M. smegmatis
B
XN
X
K
S
M. avium
65 kD Gene

FIG. 5

FIGURE 6

```
F  R  V  N  R  L  G  E  I  A  R  P  G  A  R  I  A  H  Q  G
 S  V  S  I  A  S  V  R  *  P  D  Q  A  R  G  S  R  T  K  A
  P  C  Q  S  P  R  *  D  S  P  T  R  R  A  D  R  A  P  R  R
TTCCGTGTCAATCGCCTCGGTGAGATAGCCCGACCAGGCGCGCGGATCGCGCACCAAGGC
        10        20        30        40        50        60
AAGGCACAGTTAGCGGAGCCACTCTATCGGGCTGGTCCGCGCGCCTAGCGCGTGGTTCCG
E  T  D  I  A  E  T  L  Y  G  S  W  A  R  P  D  R  V  L  A
 G  H  *  D  G  R  H  S  L  G  V  L  R  A  S  R  A  G  L  R
  R  T  L  R  R  P  S  I  A  R  G  P  A  R  I  A  C  W  P  A

A  A  Q  G  Q  Q  V  V  Q  V  M  R  G  V  F  G  H  A  Q  R
 P  R  R  V  S  R  L  C  R  L  C  V  A  F  S  A  M  H  N  A
  R  A  G  S  A  G  C  A  G  Y  A  W  R  F  R  P  C  T  T  R
GCCGCGCAGGGTCAGCAGGTTGTGCAGGTTATGCGTGGCGTTTTCGGCCATGCACAACGC
        70        80        90       100       110       120
CGGCGCGTCCCAGTCGTCCAACACGTCCAATACGCACCGCAAAAGCCGGTACGTGTTGCG
G  R  L  T  L  L  N  H  L  N  H  T  A  N  E  A  M  C  L  A
 R  A  P  D  A  P  Q  A  P  *  A  H  R  K  R  G  H  V  V  R
  A  C  P  *  C  T  T  C  T  I  R  P  T  K  P  W  A  C  R  A
```

FIGURE 6 (CONT'D)

```
A  E  A  R  E  I  E  V  H  L  R  R  G  L  G  A  R  G  H  L
 R  K  R  G  K  *  K  C  I  S  A  G  A  S  V  P  G  A  I  W
  G  S  A  G  N  R  S  A  S  P  Q  G  P  R  C  P  G  P  S  G
GCGGAAGCGCGGGAAATAGAAGTGCATCTCCGCAGGGGCCTCGGTGCCCGGGGCCATCTG
        130        140        150        160        170        180
CGCCTTCGCGCCCTTTATCTTCACGTAGAGGCGTCCCCGGAGCCACGGGCCCCGGTAGAC
R  F  R  P  F  Y  F  H  M  E  A  P  A  E  T  G  P  A  M  Q
 P  L  A  P  F  L  L  A  D  G  C  P  G  R  H  G  P  G  D  P
  S  A  R  S  I  S  T  C  R  R  L  P  R  P  A  R  P  W  R  S

E  L  D  L  H  P  V  D  G  V  G  L  P  G  L  G  D  V  D  R
 N  S  I  S  T  P  S  M  V  W  V  S  P  V  S  V  M  S  T  V
  T  R  S  P  P  R  R  W  C  G  S  P  R  S  R  *  C  R  P  S
GAACTCGATCTCCACCCCGTCGATGGTGTGGGTCTCCCCGGTCTCGGTGATGTCGACCGT
        190        200        210        220        230        240
CTTGAGCTAGAGGTGGGGCAGCTACCACACCCAGAGGGGCCAGAGCCACTACAGCTGGCA
F  E  I  E  V  G  D  I  T  H  T  E  G  T  E  T  I  D  V  T
 V  R  D  G  G  R  R  H  H  P  D  G  R  D  R  H  H  R  G  D
  S  S  R  W  G  T  S  P  T  P  R  G  P  R  P  S  T  S  R  R
```

FIGURE 6 (CONT'D)

```
R  H  D  E  R  N  L  T  G  R  Q  C  L  P  E  A  A  A  D  V
 G  T  T  S  E  T  S  P  V  D  S  V  C  P  R  P  Q  P  T  C
  A  R  R  A  K  P  H  R  S  T  V  S  A  R  G  R  S  R  R  A
CGGCACGACGAGCGAAACCTCACCGGTCGACAGTGTCTGCCCGAGGCCGCAGCCGACGTG
         250        260        270        280        290        300
GCCGTGCTGCTCGCTTTGGAGTGGCCAGCTGTCACAGACGGGCTCCGGCGTCGGCTGCAC
P  V  V  L  S  V  E  G  T  S  L  T  Q  G  L  G  C  G  V  H
 A  R  R  A  F  G  *  R  D  V  T  D  A  R  P  R  L  R  R  A
  C  S  S  R  F  R  V  P  R  C  H  R  G  S  A  A  A  S  T  G

P  P  E  T  A  R  Q  H  G  A  V  H  V  A  R  T  A  H  H  R
 P  R  R  P  R  A  N  T  V  P  Y  M  *  P  A  R  R  I  I  A
  P  G  D  R  A  P  T  R  C  R  T  C  S  P  H  G  A  S  S  P
CCCCCGGAGACCGCGCCAACACGGTGCCGTACATGTAGCCCGCACGGCGCATCATCGC
         310        320        330        340        350        360
GGGGGCCTCTGGCGCGCGGTTGTGCCACGGCATGTACATCGGGCGTGCCGCGTAGTAGCG
G  R  L  G  R  A  L  V  T  G  Y  M  Y  G  A  R  R  M  M  A
 G  P  S  R  A  G  V  R  H  R  V  H  L  G  C  P  A  D  D  G
  G  S  V  A  R  W  C  P  A  T  C  T  A  R  V  A  C  *  R  R
```

FIGURE 6 (CONT'D)

```
R  A  G  V  D  V  F  L  H  G  V  R  G  E  P  L  R  R  Q  H
 E  P  A  *  M  F  S  C  T  A  C  A  V  N  P  S  G  A  S  T
  S  R  R  R  C  F  P  A  R  R  A  R  *  T  P  P  A  P  A  P
CGAGCCGGCGTAGATGTTTTCCTGCACGGCGTGCGCGGTGAACCCCTCCGGCGCCAGCAC
         370       380       390       400       410       420
GCTCGGCCGCATCTACAAAAGGACGTGCCGCACGCGCCACTTGGGGAGGCCGCGGTCGTG
S  G  A  Y  I  N  E  Q  V  A  H  A  T  F  G  E  P  A  L  V
 L  R  R  L  H  K  G  A  R  R  A  R  H  V  G  G  A  G  A  G
  A  P  T  S  T  K  R  C  P  T  R  P  S  G  R  R  R  W  C  R

R  H  L  S  R  V  H  V  G  L  G  G  D  A  E  H  P  T  E  M
 A  T  F  P  A  S  T  S  A  W  V  V  T  P  S  T  P  P  K  *
  P  P  F  P  R  P  R  R  P  G  W  *  R  R  A  P  H  R  N  D
CGCCACCTTTCCCGCGTCCACGTCGGCCTGGGTGGTGACGCCGAGCACCCCACCGAAATG
         430       440       450       460       470       480
GCGGTGGAAAGGGCGCAGGTGCAGCCGGACCCACCACTGCGGCTCGTGGGGTGGCTTTAC
A  V  K  G  A  D  V  D  A  Q  T  T  V  G  L  V  G  G  F  H
 G  G  K  G  R  G  R  R  G  P  H  H  R  R  A  G  W  R  F  S
  W  R  E  R  T  W  T  P  R  P  P  S  A  S  C  G  V  S  I  I
```

FIGURE 6 (CONT'D)

```
I  D  M  A  V  G  V  D  D  R  D  H  G  A  V  G  S  A  V  G
 S  T  W  L  W  V  *  M  T  A  T  T  G  R  S  A  P  R  W  A
  R  H  G  C  G  C  R  *  P  R  P  R  G  G  R  L  R  G  G  R
ATCGACATGGCTGTGGGTGTAGATGACCGCGACCACGGGGCGGTCGGCTCCGCGGTGGGC
        490       500       510       520       530       540
TAGCTGTACCGACACCCACATCTACTGGCGCTGGTGCCCCGCCAGCCGAGGCGCCACCCG
D  V  H  S  H  T  Y  I  V  A  V  V  P  R  D  A  G  R  H  A
 R  C  P  Q  P  H  L  H  G  R  G  R  P  P  R  S  R  P  P  R
  S  M  A  T  P  T  S  S  R  S  W  P  A  T  P  E  A  T  P  A

A  I  Q  V  Q  R  G  G  G  H  L  G  G  H  Q  R  V  D  D  D
 R  Y  K  S  S  A  A  A  A  T  S  V  D  T  N  G  S  M  T  I
  D  T  S  P  A  R  R  R  P  P  R  W  T  P  T  G  R  *  R  S
GCGATACAAGTCCAGCGCGGCGGCGGCCACCTCGGTGGACACCAACGGGTCGATGACGAT
        550       560       570       580       590       600
CGCTATGTTCAGGTCGCGCCGCCGCCGGTGGAGCCACCTGTGGTTGCCCAGCTACTGCTA
R  Y  L  D  L  A  A  A  A  V  E  T  S  V  L  P  D  I  V  I
 S  V  L  G  A  R  R  R  G  G  R  H  V  G  V  P  R  H  R  D
  I  C  T  W  R  P  P  P  W  R  P  P  C  W  R  T  S  S  S  *
```

FIGURE 6 (CONT'D)

```
Q P S V T L N E A D I G D I E S A D L I
 S P V S P S T K L I L E I S N P R T * *
  A Q C H P Q R S * Y W R Y R I R G P D R
CAGCCCAGTGTCACCCTCAACGAAGCTGATATTGGAGATATCGAATCCGCGGACCTGATA
        610       620       630       640       650       660
GTCGGGTCACAGTGGGAGTTGCTTCGACTATAACCTCTATAGCTTAGGCGCCTGGACTAT
L G T D G E V F S I N S I D F G R V Q Y
 A W H * G * R L Q Y Q L Y R I R P G S L
  G L T V R L S A S I P S I S D A S R I S

D A R H H L V E A L F R G Q L G L P P Q
 M P G T T W * R P C F A V S W D C R H R
  C P A P P G R G P V S R S A G I A A T G
GATGCCCGGCACCACCTGGTAGAGGCCCTGTTTCGCGGTCAGCTGGGATTGCCGCCACAG
        670       680       690       700       710       720
CTACGGGCCGTGGTGGACCATCTCCGGGACAAAGCGCCAGTCGACCCTAACGGCGGTGTC
I G P V V Q Y L G Q K A T L Q S Q R W L
 H G A G G P L P G T E R D A P I A A V P
```

FIGURE 6 (CONT'D)

```
 A R C W R T S A R N R P * S P N G G C A

A G M H R C R R G T V E K R V R V V V P
 L G C T D V G A A P S R N E Y A S L S H
  W D A P M S A R H R R E T S T R R C P T
GCTGGGATGCACCGATGTCGGCGCGGCACCGTCGAGAAACGAGTACGCGTCGTTGTCCCA
         730       740       750       760       770       780
CGACCCTACGTGGCTACAGCCGCGCCGTGGCAGCTCTTTGCTCATGCGCAGCAACAGGGT
S P H V S T P A A G D L F S Y A D N D W
 Q S A G I D A R C R R S V L V R R Q G V
  P I C R H R R P V T S F R T R T T T G C

H H A T I G S L D H T R G Q R G N E S A
 T T R P S A A L I T H G D S A A M N P R
  P R D H R Q P * S H T G T A R Q * I R D
CACCACGCGACCATCGGCAGCCTTGATCACACACGGGGACAGCGCGGCAATGAATCCGCG
         790       800       810       820       830       840
GTGGTGCGCTGGTAGCCGTCGGAACTAGTGTGTGCCCCTGTCGCGCCGTTACTTAGGCGC
V V R G D A A K I V C P S L A A I F G R
 G R S W R C G Q D C V P V A R C H I R S
  W A V M P L R S * V R P C R P L S D A I
```

FIGURE 6 (CONT'D)

```
I  G  V  V  E  I  R  C  V  M  Q  R  *  R  V  F  T  V  C  R
 S  A  S  S  K  S  V  V  S  C  N  G  N  E  C  S  P  C  A  A
  R  R  R  R  N  P  L  C  H  A  T  V  T  S  V  H  R  V  P  P
ATCGGCGTCGTCGAAATCCGTTGTGTCATGCAACGGTAACGAGTGTTCACCGTGTGCCGC
        850       860       870       880       890       900
TAGCCGCAGCAGCTTTAGGCAACACAGTACGTTGCCATTGCTCACAAGTGGCACACGGCG
D  A  D  D  F  D  T  T  D  H  L  P  L  S  H  E  G  H  A  A
 R  R  R  R  F  G  N  H  *  A  V  T  V  L  T  *  R  T  G  G
  P  T  T  S  I  R  Q  T  M  C  R  Y  R  T  N  V  T  H  R  R

L  D  D  G  S  G  R  F  V  F  H  R  H  Y  I  A  T  T  T  V
 W  M  T  A  V  G  G  L  C  S  I  G  T  T  L  P  L  L  R  C
  G  *  R  Q  W  E  V  C  V  P  S  A  L  H  C  H  Y  Y  G  A
CTGGATGACGGCAGTGGGAGGTTTGTGTTCCATCGGCACTACATTGCCACTACTACGGTG
        910       920       930       940       950       960
GACCTACTGCCGTCACCCTCCAAACACAAGGTAGCCGTGATGTAACGGTGATGATGCCAC
Q  I  V  A  T  P  P  K  H  E  M  P  V  V  N  G  S  S  R  H
 P  H  R  C  H  S  T  Q  T  G  D  A  S  C  Q  W  *  *  P  A
  S  S  P  L  P  L  N  T  N  W  R  C  *  M  A  V  V  V  T  C
```

FIGURE 6 (CONT'D)

```
H  A  G  R  C  R  W  R  T  T  L  P  T  R  K  R  E  F  S  A
 T  P  V  D  A  V  G  E  P  R  Y  R  P  E  R  E  N  F  P  P
  R  R  *  M  P  L  A  N  H  A  T  D  Q  K  E  R  I  F  R  R
CACGCCGGTAGATGCCGTTGGCGAACCACGCTACCGACCAGAAAGAGAGAATTTTCCGCC
       970       980       990      1000      1010      1020
GTGCGGCCATCTACGGCAACCGCTTGGTGCGATGGCTGGTCTTTCTCTCTTAAAAGGCGG
V  G  T  S  A  T  P  S  G  R  *  R  G  S  L  S  F  K  G  G
 R  R  Y  I  G  N  A  F  W  A  V  S  W  F  S  L  I  K  R  R
  A  P  L  H  R  Q  R  V  V  S  G  V  L  F  L  S  N  E  A  A

A  P  R  P  R  A  L  L  T  R  I  L  P  K  R  S  S  M  P  M
 H  L  D  L  G  P  C  *  R  A  Y  C  R  S  G  P  Q  C  R  W
  T  *  T  S  G  P  A  N  A  H  T  A  E  A  V  L  N  A  D  G
GCACCTAGACCTCGGGCCCTGCTAACGCGCATACTGCCGAAGCGGTCCTCAATGCCGATG
      1030      1040      1050      1060      1070      1080
CGTGGATCTGGAGCCCGGGACGATTGCGCGTATGACGGCTTCGCCAGGAGTTACGGCTAC
C  R  S  R  P  G  Q  *  R  A  Y  Q  R  L  P  G  *  H  R  H
```

FIGURE 6 (CONT'D)

```
V  *  V  E  P  G  A  L  A  C  V  A  S  A  T  R  L  A  S  P
 G  L  G  R  A  R  S  V  R  M  S  G  F  R  D  E  I  G  I  S

D  R  Y  D  R  Q  R  S  T  G  *  S  V  D  *  R  S  R  *  P
 T  A  T  T  G  K  G  A  Q  G  E  A  W  T  D  G  R  G  S  R
  P  L  R  Q  A  K  E  H  R  V  K  R  G  L  T  V  A  V  A  G
GACCGCTACGACAGGCAAAGGAGCACAGGGTGAAGCGTGGACTGACGGTCGCGGTAGCCG
      1090      1100      1110      1120      1130      1140
CTGGCGATGCTGTCCGTTTCCTCGTGTCCCACTTCGCACCTGACTGCCAGCGCCATCGGC
V  A  V  V  P  L  P  A  C  P  S  A  H  V  S  P  R  P  L  R
 G  S  R  C  A  F  S  C  L  T  F  R  P  S  V  T  A  T  A  P
  R  *  S  L  C  L  L  V  P  H  L  T  S  Q  R  D  R  Y  G  S

E  P  P  F  W  S  Q  V  F  P  D  V  Q  A  T  S  R  L  Q  E
 S  R  H  S  G  R  R  S  F  R  M  F  K  Q  Q  V  D  Y  R  K
  A  A  I  L  V  A  G  L  S  G  C  S  S  N  K  S  T  T  G  S
GAGCCGCCATTCTGGTCGCAGGTCTTTCCGGATGTTCAAGCAACAAGTCGACTACAGGAA
      1150      1160      1170      1180      1190      1200
CTCGGCGGTAAGACCAGCGTCCAGAAAGGCCTACAAGTTCGTTGTTCAGCTGATGTCCTT
L  R  W  E  P  R  L  D  K  R  I  N  L  C  C  T  S  *  L  F
 A  A  M  R  T  A  P  R  E  P  H  E  L  L  L  D  V  V  P  L
  G  G  N  Q  D  C  T  K  G  S  T  *  A  V  L  R  S  C  S  A
```

FIGURE 6 (CONT'D)

```
A  V  R  P  R  P  R  Q  A  R  R  Q  A  P  A  P  P  P  G  R
 R  *  D  H  D  R  G  R  H  D  G  K  P  R  R  R  L  R  A  E
  G  E  T  T  T  A  A  G  T  T  A  S  P  G  A  A  S  G  P  K
GCGGTGAGACCACGACCGCGGCAGGCACGACGGCAAGCCCCGGCGCCGCCTCCGGGCCGA
       1210      1220      1230      1240      1250      1260
CGCCACTCTGGTGCTGGCGCCGTCCGTGCTGCCGTTCGGGGCCGCGGCGGAGGCCCGGCT
R  H  S  W  S  R  P  L  C  S  P  L  G  R  R  R  R  R  A  S
 P  S  V  V  V  A  A  P  V  V  A  L  G  P  A  A  E  P  G  F
  T  L  G  R  G  R  C  A  R  R  C  A  G  A  G  G  G  P  R  L

R  S  S  S  T  V  R  T  R  T  S  P  A  P  W  C  A  Q  P  R
 G  R  H  R  R  *  G  P  E  R  H  R  L  R  G  V  H  N  R  G
  V  V  I  D  G  K  D  Q  N  V  T  G  S  V  V  C  T  T  A  A
AGGTCGTCATCGACGGTAAGGACCAGAACGTCACCGGCTCCGTGGTGTGCACAACCGCGG
       1270      1280      1290      1300      1310      1320
TCCAGCAGTAGCTGCCATTCCTGGTCTTGCAGTGGCCGAGGCACCACACGTGTTGGCGCC
P  R  *  R  R  Y  P  G  S  R  *  R  S  R  P  T  C  L  R  P
 T  T  M  S  P  L  S  W  F  T  V  P  E  T  T  H  V  V  A  A
  D  D  D  V  T  L  V  L  V  D  G  A  G  H  H  A  C  G  R  G
```

FIGURE 6 (CONT'D)

```
P  A  M  S  T  S  R  S  A  G  R  R  P  A  L  P  P  C  S  P
 R  Q  C  Q  H  R  D  R  R  G  G  D  R  H  C  R  R  A  H  R
  G  N  V  N  I  A  I  G  G  A  A  T  G  I  A  A  V  L  T  D
CCGGCAATGTCAACATCGCGATCGGCGGGGCGGCGACCGGCATTGCCGCCGTGCTCACCG
      1330      1340      1350      1360      1370      1380
GGCCGTTACAGTTGTAGCGCTAGCCGCCCCGCCGCTGGCCGTAACGGCGGCACGAGTGGC
R  C  H  *  C  R  S  R  R  P  P  S  R  C  Q  R  R  A  *  R
 P  L  T  L  M  A  I  P  P  A  A  V  P  M  A  A  T  S  V  S
  A  I  D  V  D  R  D  A  P  R  R  G  A  N  G  G  H  E  G  V

T  A  T  L  R  R  *  S  P  L  G  S  V  T  S  T  A  S  R  W
 R  Q  P  S  G  G  E  V  R  W  A  R  *  R  Q  R  R  H  A  G
  G  N  P  P  E  V  K  S  V  G  L  G  N  V  N  G  V  T  L  G
ACGGCAACCCTCCGGAGGTGAAGTCCGTTGGGCTCGGTAACGTCAACGGCGTCACGCTGG
      1390      1400      1410      1420      1430      1440
TGCCGTTGGGAGGCCTCCACTTCAGGCAACCCGAGCCATTGCAGTTGCCGCAGTGCGACC
R  C  G  E  P  P  S  T  R  Q  A  R  Y  R  *  R  R  *  A  P
 P  L  G  G  S  T  F  D  T  P  S  P  L  T  L  P  T  V  S  P
  A  V  R  R  L  H  L  G  N  P  E  T  V  D  V  A  D  R  Q  S
```

FIGURE 6 (CONT'D)

```
D  T  R  R  A  P  D  R  V  T  P  R  Q  P  R  T  A  A  T  T
 I  H  V  G  H  R  T  G  *  R  L  G  N  Q  G  R  Q  P  L  Q
  Y  T  S  G  T  G  Q  G  N  A  S  A  T  K  D  G  S  H  Y  K
GATACACGTCGGGCACCGGACAGGGTAACGCCTCGGCAACCAAGGACGGCAGCCACTACA
       1450      1460      1470      1480      1490      1500
CTATGTGCAGCCCGTGGCCTGTCCCATTGCGGAGCCGTTGGTTCCTGCCGTCGGTGATGT
 I  C  T  P  C  R  V  P  Y  R  R  P  L  W  P  R  C  G  S  C
 Y  V  D  P  V  P  C  P  L  A  E  A  V  L  S  P  L  W  *  L
  V  R  R  A  G  S  L  T  V  G  R  C  G  L  V  A  A  V  V  L

R  S  L  G  P  L  P  G  S  T  W  P  T  R  C  H  R  *  T  S
 D  H  W  D  R  Y  R  G  R  H  G  Q  P  D  V  T  G  E  Q  V
  I  T  G  T  A  T  G  V  D  M  A  N  P  M  S  P  V  N  K  S
AGATCACTGGGACCGCTACCGGGGTCGACATGGCCAACCCGATGTCACCGGTGAACAAGT
       1510      1520      1530      1540      1550      1560
TCTAGTGACCCTGGCGATGGCCCCAGCTGTACCGGTTGGGCTACAGTGGCCACTTGTTCA
S  *  Q  S  R  *  R  P  R  C  P  W  G  S  T  V  P  S  C  T
 I  V  P  V  A  V  P  T  S  M  A  L  G  I  D  G  T  F  L  D
  D  S  P  G  S  G  P  D  V  H  G  V  R  H  *  R  H  V  L  R
```

FIGURE 6 (CONT'D)

```
R  S  K  S  R  *  P  V  P  N  L  K  R  V  D  A  G  C  E  Q
 V  R  N  R  G  D  L  F  L  T  *  S  V  S  M  R  A  V  N  S
  F  E  I  E  V  T  C  S  *  P  K  A  C  R  C  G  L  *  T  A
CGTTCGAAATCGAGGTGACCTGTTCCTAACCTAAAGCGTGTCGATGCGGGCTGTGAACAG
      1570      1580      1590      1600      1610      1620
GCAAGCTTTAGCTCCACTGGACAAGGATTGGATTTCGCACAGCTACGCCCGACACTTGTC
T  R  F  R  P  S  R  N  R  V  *  L  T  D  I  R  A  T  F  L
 N  S  I  S  T  V  Q  E  *  G  L  A  H  R  H  P  S  H  V  A
  E  F  D  L  H  G  T  G  L  R  F  R  T  S  A  P  Q  S  C  R

R  V  G  A  G  Q  S  G  L  A  R  R  R  F  E  R  L  P  S  V
 A  S  E  P  G  S  Q  A  *  R  G  D  D  S  S  G  C  H  P  S
  R  R  S  R  A  V  R  P  S  A  A  T  I  R  A  V  A  I  R  Q
CGCGTCGGAGCCGGGCAGTCAGGCCTAGCGCGGCGACGATTCGAGCGGTTGCCATCCGTC
      1630      1640      1650      1660      1670      1680
GCGCAGCCTCGGCCCGTCAGTCCGGATCGCGCCGCTGCTAAGCTCGCCAACGGTAGGCAG
A  D  S  G  P  L  *  A  *  R  P  S  S  E  L  P  Q  W  G  D
 R  R  L  R  A  T  L  G  L  A  A  V  I  R  A  T  A  M  R  *
  T  P  A  P  C  D  P  R  A  R  R  R  N  S  R  N  G  D  T  L
```

FIGURE 6 (CONT'D)

```
K  W  Q  P  H  R  K  L  G  I  S  G  *  A  T  H  G  D  R  S
 S  G  N  R  T  A  N  S  V  Y  P  G  E  L  L  T  V  I  V  P
  V  A  T  A  P  Q  T  R  Y  I  R  V  S  Y  S  R  *  S  F  F
AAGTGGCAACCGCACCGCAAACTCGGTATATCCGGGTGAGCTACTCACGGTGATCGTTCC
       1690      1700      1710      1720      1730      1740
TTCACCGTTGGCGTGGCGTTTGAGCCATATAGGCCCACTCGATGAGTGCCACTAGCAAGG
L  P  L  R  V  A  F  E  T  Y  G  P  S  S  S  V  T  I  T  G
 T  A  V  A  G  C  V  R  Y  I  R  T  L  *  E  R  H  D  N  R
  H  C  G  C  R  L  S  P  I  D  P  H  A  V  *  P  S  R  E  T

V  V  R  L  D  H  S  G  D  D  R  Q  A  E  P  G  A  T  G  L
 L  C  A  L  T  T  A  E  T  I  A  R  P  S  P  V  L  P  A  W
  C  A  P  *  P  Q  R  R  R  S  P  G  R  A  R  C  Y  R  L  G
GTTGTGCGCCTTGACCACAGCGGAGACGATCGCCAGGCCGAGCCCGGTGCTACCGGCTTG
       1750      1760      1770      1780      1790      1800
CAACACGCGGAACTGGTGTCGCCTCTGCTAGCGGTCCGGCTCGGGCCACGATGGCCGAAC
N  H  A  K  V  V  A  S  V  I  A  L  G  L  G  T  S  G  A  Q
 Q  A  G  Q  G  C  R  L  R  D  G  P  R  A  R  H  *  R  S  P
  T  R  R  S  W  L  P  S  S  R  W  A  S  G  P  A  V  P  K  A
```

FIGURE 6 (CONT'D)

```
A  G  P  *  R  I  A  A  G  E  P  L  E  N  L  G  L  Q  R  G
 R  D  R  D  V  S  P  R  A  N  R  S  K  T  S  D  C  S  A  A
  G  T  V  T  Y  R  R  G  R  T  A  R  K  P  R  T  A  A  R  P
GCGGGACCGTGACGTATCGCCGCGGGCGAACCGCTCGAAAACCTCGGACTGCAGCGCGGC
        1810      1820      1830      1840      1850      1860
CGCCCTGGCACTGCATAGCGGCGCCCGCTTGGCGAGCTTTTGGAGCCTGACGTCGCGCCG
R  S  R  S  T  D  G  R  A  F  R  E  F  V  E  S  Q  L  A  A
 P  V  T  V  Y  R  R  P  R  V  A  R  F  G  R  V  A  A  R  G
  P  G  H  R  I  A  A  P  S  G  S  S  F  R  P  S  C  R  P  R

R  N  T  R  P  I  V  D  H  L  Q  H  D  V  R  R  P  G  A  Q
 G  I  P  G  P  L  S  I  T  C  S  T  T  C  V  G  P  V  L  K
  E  Y  P  A  H  C  R  S  P  A  A  R  R  A  S  A  R  C  S  S
CGGAATACCCGGCCCATTGTCGATCACCTGCAGCACGACGTGCGTCGGCCCGGTGCTCAA
        1870      1880      1890      1900      1910      1920
GCCTTATGGGCCGGGTAACAGCTAGTGGACGTCGTGCTGCACGCAGCCGGCCACGAGTT
P  I  G  P  G  N  D  I  V  Q  L  V  V  H  T  P  G  T  S  L
 S  Y  G  A  W  Q  R  D  G  A  A  R  R  A  D  A  R  H  E  L
  F  V  R  G  M  T  S  *  R  C  C  S  T  R  R  G  P  A  *  A
```

FIGURE 6 (CONT'D)

```
A  R  R  H  D  R  A  G  T  G  V  H  A  G  V  G  Q  Q  V  G
 R  V  V  T  I  V  P  G  P  V  C  T  R  A  L  A  S  R  L  V
  A  S  S  R  S  C  R  D  R  C  A  R  G  R  W  P  A  G  W  S
GCGCGTCGTCACGATCGTGCCGGGACCGGTGTGCACGCGGGCGTTGGCCAGCAGGTTGGT
      1930      1940      1950      1960      1970      1980
CGCGCAGCAGTGCTAGCACGGCCCTGGCCACACGTGCGCCCGCAACCGGTCGTCCAACCA
R  T  T  V  I  T  G  P  G  T  H  V  R  A  N  A  L  L  N  T
 A  D  D  R  D  H  R  S  R  H  A  R  P  R  Q  G  A  P  Q  D
  R  R  *  S  R  A  P  V  P  T  C  A  P  T  P  W  C  T  P  *

H  H  L  V  Q  P  C  R  I  T  R  D  D  H  R  F  G  G  Q  V
 T  T  W  C  N  R  A  A  S  P  G  M  T  T  G  S  G  G  R  S
  P  P  G  A  T  V  P  H  H  P  G  *  P  P  V  R  G  A  G  R
CACCACCTGGTGCAACCGTGCCGCATCACCCGGGATGACCACCGGTTCGGGGGCAGGTC
      1990      2000      2010      2020      2030      2040
GTGGTGGACCACGTTGGCACGGCGTAGTGGGCCCTACTGGTGGCCAAGCCCCCGTCCAG
V  V  Q  H  L  R  A  A  D  G  P  I  V  V  P  E  P  P  L  D
 G  G  P  A  V  T  G  C  *  G  P  H  G  G  T  R  P  A  P  R
  W  R  T  C  G  H  R  M  V  R  S  S  W  R  N  P  P  C  T  S
```

FIGURE 6 (CONT'D)

```
E  R  P  L  V  I  W  S  G  N  M  S  V  A  D  R  V  N  R  K
 S  A  H  W  *  S  G  P  A  T  *  A  S  L  T  A  S  T  A  S
  A  P  T  G  D  L  V  R  Q  H  E  R  R  *  P  R  Q  P  Q  A
GAGCGCCCACTGGTGATCTGGTCCGGCAACATGAGCGTCGCTGACCGCGTCAACCGCAAG
      2050      2060      2070      2080      2090      2100
CTCGCGGGTGACCACTAGACCAGGCCGTTGTACTCGCAGCGACTGGCGCAGTTGGCGTTC
L  A  W  Q  H  D  P  G  A  V  H  A  D  S  V  A  D  V  A  L
 A  G  V  P  S  R  T  R  C  C  S  R  R  Q  G  R  *  G  C  A
  R  G  S  T  I  Q  D  P  L  M  L  T  A  S  R  T  L  R  L  G

P  R  H  V  H  R  S  A  F  Q  R  P  P  R  V  Q  T  R  Q  Q
 R  D  M  S  T  G  P  R  S  S  G  R  P  E  S  R  R  A  S  S
  A  T  C  P  P  V  R  V  P  A  A  A  P  S  P  D  A  P  A  A
CCGCGACATGTCCACCGGTCCGCGTTCCAGCGGCCGCCCCGAGTCCAGACGCGCCAGCAG
      2110      2120      2130      2140      2150      2160
GGCGCTGTACAGGTGGCCAGGCGCAAGGTCGCCGGCGGGGCTCAGGTCTGCGCGGTCGTC
R  S  M  D  V  P  G  R  E  L  P  R  G  S  D  L  R  A  L  L
 A  V  H  G  G  T  R  T  G  A  A  A  G  L  G  S  A  G  A  A
  R  C  T  W  R  D  A  N  W  R  G  G  R  T  W  V  R  W  C  C
```

FIGURE 6 (CONT'D)

```
Q  Q  V  L  D  E  T  C  Y  P  L  G  L  R  C  H  P  A  H  R
 S  R  S  S  T  R  R  V  I  R  S  V  S  D  A  T  R  L  I  A
  A  G  P  R  R  D  V  L  S  A  R  S  P  M  P  P  G  S  S  R
CAGCAGGTCCTCGACGAGACGTGTTATCCGCTCGGTCTCCGATGCCACCCGGCTCATCGC
        2170      2180      2190      2200      2210      2220
GTCGTCCAGGAGCTGCTCTGCACAATAGGCGAGCCAGAGGCTACGGTGGGCCGAGTAGCG
L  L  D  E  V  L  R  T  I  R  E  T  E  S  A  V  R  S  M  A
 A  P  G  R  R  S  T  N  D  A  R  D  G  I  G  G  P  E  D  R
  C  T  R  S  S  V  H  *  G  S  P  R  R  H  W  G  A  *  R  T

V  C  D  G  L  G  I  V  P  Y  P  L  R  Q  F  R  V  T  T  D
 C  A  T  A  S  G  S  S  P  I  R  C  V  N  S  V  *  P  R  I
  V  R  R  P  R  D  R  P  L  S  A  A  S  I  P  C  N  H  G  S
GTGTGCGACGGCCTCGGGATCGTCCCCTATCCGCTGCGTCAATTCCGTGTAACCACGGAT
        2230      2240      2250      2260      2270      2280
CACACGCTGCCGGAGCCCTAGCAGGGGATAGGCGACGCAGTTAAGGCACATTGGTGCCTA
H  A  V  A  E  P  D  D  G  I  R  Q  T  L  E  T  Y  G  R  I
 T  R  R  G  R  S  R  G  R  D  A  A  D  I  G  H  L  W  P  D
  H  S  P  R  P  I  T  G  *  G  S  R  *  N  R  T  V  V  S  R
```

FIGURE 6 (CONT'D)

```
R  R  K  G  S  S  Q  F  M  T  G  I  G  N  E  L  A  H  T  G
 A  A  R  G  V  R  S  S  *  L  A  S  A  T  N  W  R  T  R  V
  P  Q  G  E  F  A  V  H  D  W  H  R  Q  R  T  G  A  H  G  F
CGCCGCAAGGGGAGTTCGCAGTTCATGACTGGCATCGGCAACGAACTGGCGCACACGGGT
       2290      2300      2310      2320      2330      2340
GCGGCGTTCCCCTCAAGCGTCAAGTACTGACCGTAGCCGTTGCTTGACCGCGTGTGCCCA
 A  A  L  P  T  R  L  E  H  S  A  D  A  V  F  Q  R  V  R  T
 G  C  P  S  N  A  T  *  S  Q  C  R  C  R  V  P  A  C  P  N
  R  L  P  L  E  C  N  M  V  P  M  P  L  S  S  A  C  V  P  K

F  T  G  L  P  R  R  Q  C  G  S  D  V  V  E  H  P  V  E  R
 S  L  A  C  R  A  D  S  A  A  A  M  W  S  S  I  R  L  S  A
  H  W  P  A  A  P  T  V  R  Q  R  C  G  R  A  S  G  *  A  P
TTCACTGGCCTGCCGCGCCGACAGTGCGGCAGCGATGTGGTCGAGCATCCGGTTGAGCGC
       2350      2360      2370      2380      2390      2400
AAGTGACCGGACGGCGCGGCTGTCACGCCGTCGCTACACCAGCTCGTAGGCCAACTCGCG
E  S  A  Q  R  A  S  L  A  A  A  I  H  D  L  M  R  N  L  A
 *  Q  G  A  A  G  V  T  R  C  R  H  P  R  A  D  P  Q  A  G
  V  P  R  G  R  R  C  H  P  L  S  T  T  S  C  G  T  S  R  R
```

FIGURE 6 (CONT'D)

```
R  P  E  L  P  H  L  G  G  G  V  C  V  R  F  G  H  P  D  R
 D  P  S  C  P  T  S  V  E  G  F  A  S  G  S  G  T  R  T  G
  T  R  V  A  P  P  R  W  R  G  L  R  Q  V  R  A  P  G  P  V
CGACCCGAGTTGCCCCACCTCGGTGGAGGGGTTTGCGTCAGGTTCGGGCACCCGGACCGG
        2410      2420      2430      2440      2450      2460
GCTGGGCTCAACGGGGTGGAGCCACCTCCCCAAACGCAGTCCAAGCCCGTGGCCTGGCC
S  G  L  Q  G  V  E  T  S  P  N  A  D  P  E  P  V  R  V  P
 V  R  T  A  G  G  R  H  L  P  K  R  *  T  R  A  G  P  G  T
  G  S  N  G  W  R  P  P  P  T  Q  T  L  N  P  C  G  S  R  Y

*  L  D  L  A  A  I  Q  R  *  V  D  D  F  A  R  G  L  R  D
 S  L  T  S  P  R  S  N  G  R  S  T  T  S  L  A  V  C  A  T
  A  *  P  R  R  D  P  T  V  G  R  R  L  R  S  R  F  A  R  P
TAGCTTGACCTCGCCGCGATCCAACGGTAGGTCGACGACTTCGCTCGCGGTTTGCGCGAC
        2470      2480      2490      2500      2510      2520
ATCGAACTGGAGCGGCGCTAGGTTGCCATCCAGCTGCTGAAGCGAGCGCCAAACGCGCTG
L  K  V  E  G  R  D  L  P  L  D  V  V  E  S  A  T  Q  A  V
 A  Q  G  R  R  S  G  V  T  P  R  R  S  R  E  R  N  A  R  G
  S  S  R  A  A  I  W  R  Y  T  S  S  K  A  R  P  K  R  S  R
```

FIGURE 6 (CONT'D)

```
R  R  N  G  A  S  A  R  L  M  M  T  I  P  A  V  V  A  A  T
 A  A  T  A  P  A  P  A  *  *  *  R  F  R  R  S  S  R  R  P
  P  Q  R  R  Q  R  P  L  D  D  D  D  S  G  G  R  R  G  D  Q
CGCCGCAACGGCGCCAGCGCCCGCTTGATGATGACGATTCCGGCGGTCGTCGCGGCGACC
      2530      2540      2550      2560      2570      2580
GCGGCGTTGCCGCGGTCGCGGGCGAACTACTACTGCTAAGGCCGCCAGCAGCGCCGCTGG
A  A  V  A  G  A  G  A  Q  H  H  R  N  R  R  D  D  R  R  G
 G  C  R  R  W  R  G  S  S  S  S  S  E  P  P  R  R  P  S  W
  R  L  P  A  L  A  R  K  I  I  V  I  G  A  T  T  A  A  V  L

N  A  I  T  V  T  I  P  K  M  I  S  I  C  N  I  V  A  S  T
 T  Q  S  P  *  R  F  R  K  *  S  A  S  A  T  S  W  R  R  R
  R  N  H  R  D  D  S  E  N  D  Q  H  L  Q  H  R  G  V  D  V
AACGCAATCACCGTGACGATTCCGAAAATGATCAGCATCTGCAACATCGTGGCGTCGACG
      2590      2600      2610      2620      2630      2640
TTGCGTTAGTGGCACTGCTAAGGCTTTTACTAGTCGTAGACGTTGTAGCACCGCAGCTGC
V  C  D  G  H  R  N  R  F  H  D  A  D  A  V  D  H  R  R  R
 R  L  *  R  S  S  E  S  F  S  *  C  R  C  C  R  P  T  S  T
  A  I  V  T  V  I  G  F  I  I  L  M  Q  L  M  T  A  D  V  N

L  P  I  D  R  P  V  T  M  T  S  C  P  F  R  L  G  A  A  S
 C  P  S  T  G  R  *  R  *  R  R  A  R  F  G  S  E  R  P  A
  A  H  R  Q  A  G  D  D  D  V  V  P  V  S  A  R  S  G  Q  H
TTGCCCATCGACAGGCCGGTGACGATGACGTCGTGCCCGTTTCGGCTCGGAGCGGCCAGC
```

FIGURE 7

```
  1 GAA TTC CAA CCG TCG GTG CAG ATC CAG GTC TAT CAG GGG GAG CGT GAG  48
    Glu Phe Gln Pro Ser Val Gln Ile Gln Val Tyr Gln Gly Glu Arg Glu

 49 ATC GCC GCG CAC AAC AAG TTG CTC GGG TCC TTC GAG CTG ACC GGC ATC  96
    Ile Ala Ala His Asn Lys Leu Leu Gly Ser Phe Glu Leu Thr Gly Ile

 97 CCG CCG GCG CCG CGG GGG ATT CCG CAG ATC GAG GTC ACT TTC GAC ATC 144
    Pro Pro Ala Pro Arg Gly Ile Pro Gln Ile Glu Val Thr Phe Asp Ile

145 GAC GCC AAC GGC ATT GTG CAC GTC ACC GCC AAG GAC AAG GGC ACC GGC 192
    Asp Ala Asn Gly Ile Val His Val Thr Ala Lys Asp Lys Gly Thr Gly
```

FIGURE 7 (CONT'D)

```
193  AAG GAG AAC ACG ATC CGA ATC CAG GAA GGC TCG GGC CTG TCC AAG GAA  240
     Lys Glu Asn Thr Ile Arg Ile Gln Glu Gly Ser Gly Leu Ser Lys Glu

241  GAC ATT GAC CGC ATG ATC AAG GAC GCC GAA GCG CAC GCC GAG GAG GAT  288
     Asp Ile Asp Arg MET Ile Lys Asp Ala Glu Ala His Ala Glu Glu Asp

289  CGC AAG CGT CGC GAG GAG GCC GAT GTT CGT AAT CAA GCC GAG ACA TTG  336
     Arg Lys Arg Arg Glu Glu Ala Asp Val Arg Asn Gln Ala Glu Thr Leu

337  GTC TAC CAG ACG GAG AAG TTC GTC AAA GAA CAG CGT GAG GCC GAG GGT  384
     Val Tyr Gln Thr Glu Lys Phe Val Lys Glu Gln Arg Glu Ala Glu Gly
```

FIGURE 7 (CONT'D)

```
385  GGT TCG AAG GTA CCT GAA GAC ACG CTG AAC AAG GTT GAT GCC GCG GTG  432
     Gly Ser Lys Val Pro Glu Asp Thr Leu Asn Lys Val Asp Ala Ala Val

433  GCG GAA GCG GAA GGC GGC ACT TGG CGG ATC GGA TAT TTC GGC CAT CAA  480
     Ala Glu Ala Glu Gly Gly Thr Trp Arg Ile Gly Tyr Phe Gly His Gln

481  GTC GGC GAT GGA GAA GCT GGG CCA GGA GTC GCA GGC TCT GGG GCA AGC  528
     Val Gly Asp Gly Glu Ala Gly Pro Gly Val Ala Gly Ser Gly Ala Ser

529  GAT CTA CGA AGC AGC TCA GGC TGC GTC ACA GGC CAC TGG CGC TGC CCA  576
     Asp Leu Arg Ser Ser Ser Gly Cys Val Thr Gly His Trp Arg Cys Pro

577  CCC CGG CGG CGA GCC GGG CGG TGC CCA CCC CGG CTC GGC   615
     Pro Arg Arg Arg Ala Gly Arg Cys Pro Pro Arg Leu Gly
```

FIGURE 8

```
5' TCGAACGAGGGGCGTGACCCGGTGCGGGGCTTCTTGCACTCGGCATAGGCGAGTGCTAAG
3' AGCTTGCTCCCCGCACTGGGCCACGCCCCGAAGAACGTGAGCCGTATCCGCTCACGATTC
           10        20        30        40        50        60

   AATAACGTTGGCACTCGCGACCGGTGAGTGCTAGGTCGGGACGGTGAGGCCAGGCCCGTC
   TTATTGCAACCGTGAGCGCTGGCCACTCACGATCCAGCCCTGCCACTCCGGTCCGGGCAG
           70        80        90       100       110       120

   GTCGCAGCGAGTGGCAGCGAGGACAACTTGAGCCGTCCGTCGCGGGCACTGCGCCCGGCC
   CAGCGTCGCTCACCGTCGCTCCTGTTGAACTCGGCAGGCAGCGCCCGTGACGCGGGCCGG
          130       140       150       160       170       180

            *  R  G  C  R  H  P  V  T  P  V  S  S  P  I  R  R
   AGCGTAAGTAGCGGGGTTGCCGTCACCCGGTGACCCCCGTTTCATCCCCGATCCGGAGGA
   TCGCATTCATCGCCCCAACGGCAGTGGGCCACTGGGGGCAAAGTAGGGGCTAGGCCTCCT
          190       200       210       220       230       240

   N  H  F  A  M  A  K  T  I  A  Y  D  E  E  A  R  R  G  L  E
   ATCACTTCGCAATGGCCAAGACAATTGCGTACGACGAAGAGGCCCGTCGCGGCCTCGAGC
   TAGTGAAGCGTTACCGGTTCTGTTAACGCATGCTGCTTCTCCGGGCAGCGCCGGAGCTCG
          250       260       270       280       290       300

   R  G  L  N  A  L  A  D  A  V  K  V  T  L  G  P  K  G  R  N
   GGGGCTTGAACGCCCTCGCCGATGCGGTAAAGGTGACATTGGGCCCCAAGGGCCGCAACG
   CCCCGAACTTGCGGGAGCGGCTACGCCATTTCCACTGTAACCCGGGGTTCCCGGCGTTGC
          310       320       330       340       350       360

   V  V  L  E  K  K  W  G  A  P  T  I  T  N  D  G  V  S  I  A
   TCGTCCTGGAAAAGAAGTGGGGTGCCCCCACGATCACCAACGATGGTGTGTCCATCGCCA
   AGCAGGACCTTTTCTTCACCCCACGGGGGTGCTAGTGGTTGCTACCACACAGGTAGCGGT
          370       380       390       400       410       420
```

FIGURE 8 (CONT'D)

```
K  E  I  E  L  E  D  P  Y  E  K  I  G  A  E  L  V  K  E  V
AGGAGATCGAGCTGGAGGATCCGTACGAGAAGATCGGCGCCGAGCTGGTCAAAGAGGTAG
TCCTCTAGCTCGACCTCCTAGGCATGCTCTTCTAGCCGCGGCTCGACCAGTTTCTCCATC
        430       440       450       460       470       480

A  K  K  T  D  D  V  A  G  D  G  T  T  T  A  T  V  L  A  Q
CCAAGAAGACCGATGACGTCGCCGGTGACGGCACCACGACGGCCACCGTGCTGGCCCAGG
GGTTCTTCTGGCTACTGCAGCGGCCACTGCCGTGGTGCTGCCGGTGGCACGACCGGGTCC
        490       500       510       520       530       540

A  L  V  R  E  G  L  R  N  V  A  A  G  A  N  P  L  G  L  K
CGTTGGTTCGCGAGGGCCTGCGCAACGTCGCGGCCGGCGCCAACCCGCTCGGTCTCAAAC
GCAACCAAGCGCTCCCGGACGCGTTGCAGCGCCGGCCGCGGTTGGGCGAGCCAGAGTTTG
        550       560       570       580       590       600

R  G  I  E  K  A  V  E  K  V  T  E  T  L  L  K  G  A  K  E
GCGGCATCGAAAAGGCCGTGGAGAAGGTCACCGAGACCCTGCTCAAGGGCGCCAAGGAGG
CGCCGTAGCTTTTCCGGCACCTCTTCCAGTGGCTCTGGGACGAGTTCCCGCGGTTCCTCC
        610       620       630       640       650       660

V  E  T  K  E  Q  I  A  A  T  A  A  I  S  A  G  D  Q  S  I
TCGAGACCAAGGAGCAGATTGCGGCCACCGCAGCGATTTCGGCGGGTGACCAGTCCATCG
AGCTCTGGTTCCTCGTCTAACGCCGGTGGCGTCGCTAAAGCCGCCCACTGGTCAGGTAGC
        670       680       690       700       710       720

G  D  L  I  A  E  A  M  D  K  V  G  N  E  G  V  I  T  V  E
GTGACCTGATCGCCGAGGCGATGGACAAGGTGGGCAACGAGGGCGTCATCACCGTCGAGG
CACTGGACTAGCGGCTCCGCTACCTGTTCCACCCGTTGCTCCCGCAGTAGTGGCAGCTCC
        730       740       750       760       770       780
```

FIGURE 8 (CONT'D)

```
E  S  N  T  F  G  L  Q  L  E  L  T  E  G  M  R  F  D  K  G
AGTCCAACACCTTTGGGCTGCAGCTCGAGCTCACCGAGGGTATGCGGTTCGACAAGGGCT
TCAGGTTGTGGAAACCCGACGTCGAGCTCGAGTGGCTCCCATACGCCAAGCTGTTCCCGA
       790        800        810        820        830        840

Y  I  S  G  Y  F  V  T  D  P  E  R  Q  E  A  V  L  E  D  P
ACATCTCGGGGTACTTCGTGACCGACCCGGAGCGTCAGGAGGCGGTCCTGGAGGACCCCT
TGTAGAGCCCCATGAAGCACTGGCTGGGCCTCGCAGTCCTCCGCCAGGACCTCCTGGGGA
       850        860        870        880        890        900

Y  I  L  L  V  S  S  K  V  S  T  V  K  D  L  L  P  L  L  E
ACATCCTGCTGGTCAGCTCCAAGGTGTCCACTGTCAAGGATCTGCTGCCGCTGCTCGAGA
TGTAGGACGACCAGTCGAGGTTCCACAGGTGACAGTTCCTAGACGACGGCGACGAGCTCT
       910        920        930        940        950        960

K  V  I  G  A  G  K  P  L  L  I  I  A  E  D  V  E  G  E  A
AGGTCATCGGAGCCGGTAAGCCGCTGCTGATCATCGCCGAGGACGTCGAGGGCGAGGCGC
TCCAGTAGCCTCGGCCATTCGGCGACGACTAGTAGCGGCTCCTGCAGCTCCCGCTCCGCG
       970        980        990       1000       1010       1020

L  S  T  L  V  V  N  K  I  R  G  T  F  K  S  V  A  V  K  A
TGTCCACCCTGGTCGTCAACAAGATCCGCGGCACCTTCAAGTCGGTGGCGGTCAAGGCTC
ACAGGTGGGACCAGCAGTTGTTCTAGGCGCCGTGGAAGTTCAGCCACCGCCAGTTCCGAG
      1030       1040       1050       1060       1070       1080

P  G  F  G  D  R  R  K  A  M  L  Q  D  M  A  I  L  T  G  G
CCGGCTTCGGCGACCGCCGCAAGGCGATGCTGCAGGATATGGCCATTCTCACCGGTGGTC
GGCCGAAGCCGCTGGCGGCGTTCCGCTACGACGTCCTATACCGGTAAGAGTGGCCACCAG
      1090       1100       1110       1120       1130       1140

Q  V  I  S  E  E  V  G  L  T  L  E  N  A  D  L  S  L  L  G
AGGTGATCAGCGAAGAGGTCGGCCTGACGCTGGAGAACGCCGACCTGTCGCTGCTAGGCA
TCCACTAGTCGCTTCTCCAGCCGGACTGCGACCTCTTGCGGCTGGACAGCGACGATCCGT
      1150       1160       1170       1180       1190       1200
```

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FIGURE 8 (CONT'D)

```
K  A  R  K  V  V  V  T  K  D  E  T  T  I  V  E  G  A  G  D
AGGCCCGCAAGGTCGTGGTCACCAAGGACGAGACCACCATCGTCGAGGGCGCCGGTGACA
TCCGGGCGTTCCAGCACCAGTGGTTCCTGCTCTGGTGGTAGCAGCTCCCGCGGCCACTGT
        1210      1220      1230      1240      1250      1260

T  D  A  I  A  G  R  V  A  Q  I  R  Q  E  I  E  N  S  D  S
CCGACGCCATCGCCGGACGAGTGGCCCAGATCCGCCAGGAGATCGAGAACAGCGACTCCG
GGCTGCGGTAGCGGCCTGCTCACCGGGTCTAGGCGGTCCTCTAGCTCTTGTCGCTGAGGC
        1270      1280      1290      1300      1310      1320

D  Y  D  R  E  K  L  Q  E  R  L  A  K  L  A  G  G  V  A  V
ACTACGACCGTGAGAAGCTGCAGGAGCGGCTGGCCAAGCTGGCCGGTGGTGTCGCGGTGA
TGATGCTGGCACTCTTCGACGTCCTCGCCGACCGGTTCGACCGGCCACCACAGCGCCACT
        1330      1340      1350      1360      1370      1380

I  K  A  G  A  A  T  E  V  E  L  K  E  R  K  H  R  I  E  D
TCAAGGCCGGTGCCGCCACCGAGGTCGAACTCAAGGAGCGCAAGCACCGCATCGAGGATG
AGTTCCGGCCACGGCGGTGGCTCCAGCTTGAGTTCCTCGCGTTCGTGGCGTAGCTCCTAC
        1390      1400      1410      1420      1430      1440

A  V  R  N  A  K  A  A  V  E  E  G  I  V  A  G  G  G  V  T
CGGTTCGCAATGCCAAGGCCGCCGTCGAGGAGGGCATCGTCGCCGGTGGGGGTGTGACGC
GCCAAGCGTTACGGTTCCGGCGGCAGCTCCTCCCGTAGCAGCGGCCACCCCCACACTGCG
        1450      1460      1470      1480      1490      1500

L  L  Q  A  A  P  T  L  D  E  L  K  L  E  G  D  E  A  T  G
TGTTGCAAGCGGCCCCGACCCTGGACGAGCTGAAGCTCGAAGGCGACGAGGCGACCGGCG
ACAACGTTCGCCGGGGCTGGGACCTGCTCGACTTCGAGCTTCCGCTGCTCCGCTGGCCGC
        1510      1520      1530      1540      1550      1560

A  N  I  V  K  V  A  L  E  A  P  L  K  Q  I  A  F  N  S  G
CCAACATCGTGAAGGTGGCGCTGGAGGCCCCGCTGAAGCAGATCGCCTTCAACTCCGGGC
GGTTGTAGCACTTCCACCGCGACCTCCGGGGCGACTTCGTCTAGCGGAAGTTGAGGCCCG
        1570      1580      1590      1600      1610      1620
```

FIGURE 8 (CONT'D)

```
L  E  P  G  V  V  A  E  K  V  R  N  L  P  A  G  H  G  L  N
TGGAGCCGGGCGTGGTGGCCGAGAAGGTGCGCAACCTGCCGGCTGGCCACGGACTGAACG
ACCTCGGCCCGCACCACCGGCTCTTCCACGCGTTGGACGGCCGACCGGTGCCTGACTTGC
       1630      1640      1650      1660      1670      1680

A  Q  T  G  V  Y  E  D  L  L  A  A  G  V  A  D  P  V  K  V
CTCAGACCGGTGTCTACGAGGATCTGCTCGCTGCCGGCGTTGCTGACCCGGTCAAGGTGA
GAGTCTGGCCACAGATGCTCCTAGACGAGCGACGGCCGCAACGACTGGGCCAGTTCCACT
       1690      1700      1710      1720      1730      1740

T  R  S  A  L  Q  N  A  A  S  I  A  G  L  F  L  T  T  E  A
CCCGTTCGGCGCTGCAGAATGCGGCGTCCATCGCGGGGCTGTTCCTGACCACCGAGGCCG
GGGCAAGCCGCGACGTCTTACGCCGCAGGTAGCGCCCCGACAAGGACTGGTGGCTCCGGC
       1750      1760      1770      1780      1790      1800

V  V  A  D  K  P  E  K  E  K  A  S  V  P  G  G  G  D  M  G
TCGTTGCCGACAAGCCGGAAAAGGAGAAGGCTTCCGTTCCCGGTGGCGGCGACATGGGTG
AGCAACGGCTGTTCGGCCTTTTCCTCTTCCGAAGGCAAGGGCCACCGCCGCTGTACCCAC
       1810      1820      1830      1840      1850      1860

G  M  D  F  *
GCATGGATTTCTGACCCCGGCGAGAAGTCGCAGCGAGGAGCCCGGTCCCTTTGTGGGGCC
CGTACCTAAAGACTGGGGCCGCTCTTCAGCGTCGCTCCTCGGGCCAGGGAAACACCCCGG
       1870      1880      1890      1900      1910      1920

GGGCTCCTCTGGTTGGGAGCTACGGTACCGAGAACACCACGCAGTCGTGTAGGCAACCTT
CCCGAGGAGACCAACCCTCGATGCCATGGCTCTTGTGGTGCGTCAGCACATCCGTTGGAA
       1930      1940      1950      1960      1970      1980

TGGCCGCTGTGGGCGAGTCGGGGGCCGCGTCTCGGTGCAGCAGCGCGCGGATGGGTACGA
ACCGGCGACACCCGCTCAGCCCCCGGCGCAGAGCCACGTCGTCGCGCGCCTACCCATGCT
       1990      2000      2010      2020      2030      2040
```

FIGURE 8 (CONT'D)

```
CACCGCAGCGGGCGGTGTCGTCATCGGGGCCTGCGTCCGACGCCTGGGCACGGCCGTCGA
GTGGCGTCGCCCGCCACAGCAGTAGCCCCGGACGCAGGCTGCGGACCCGTGCCGGCAGCT
      2050      2060      2070      2080      2090      2100

CGATCAGCGAGTAGCCGCTAGGATCGGATGGCGGCCACAACAGGGTGACTTCGCTGCGGT
GCTAGTCGCTCATCGGCGATCCTAGCCTACCGCCGGTGTTGTCCCACTGAAGCGACGCCA
      2110      2120      2130      2140      2150      2160

GGGCCAGGTTTTGCCGCGTACGACCCCCGATCAGGCCGACGTCGACCACTGCCCGGGGTC
CCCGGTCCAAAACGGCGCATGCTGGGGGCTAGTCCGGCTGCAGCTGGTGACGGGCCCCAG
      2170      2180      2190      2200      2210      2220

CATCGGGGCCGTCGGGGAGTTCGCGCAGCACCGGCTCGACTGCCACCGTGTGCACGCGAT
GTAGCCCCGGCAGCCCCTCAAGCGCGTCGTGGCCGAGCTGACGGTGGCACACGTGCGCTA
      2230      2240      2250      2260      2270      2280

      2290      2300      2310      2320      2330      2340
GGCCATCATCGACGGTGATCAGGTAAGCGAACGGGTAGTCGGGCAAGGCGGCGGCCAGCC
CCGGTAGTAGCTGCCACTAGTCCATTCGCTTGCCCATCAGCCCGTTCCGCCGCCGGTCGG

      2360      2360      2370      2380      2390      2400
GTTTGAGGTCTACCTTTTTGGCACCCACGGATTCGAGGATAGGCGCCCGATGTGTTACTC
CAAACTCCAGATGGAAAAACCGTGGGTGCCTAAGCTCCTATCCGCGGGCTACACAATGAG

      2410      2420      2430      2440      2450      2460
CGAACCGACCGGCTGCCCGATCCGCGGGCTGGCGTAGGCGGATTCGCGGTCGGGGCTCGG
GCTTGGCTGGCCGACGGGCTAGGCGCCCGACCGCATCCGCCTAAGCGCCAGCCCCGAGCC
 S  G  V  P  Q  G  I  R  P  S  A  Y  A  S  E  R  D  P  S  P
                                                         *  E
      2470      2480      2490      2500      2510      2520
GTAGAAGTTCGACTTGGGGATGCCGGAGCCGGGGGTACTCGGCTCACGCACGGCGGTATT
CATCTTCAAGCTGAACCCCTACGGCCTCGGCCCCCATGAGCCGAGTGCGTGCCGCCATAA
 Y  F  N  S  K  P  I  G  S  G  P  T  S  P  E  R  V  A  T  N
```

FIGURE 8 (CONT'D)

```
       2530      2540      2550      2560      2570      2580
CCGCAAGCCCGAGTCGTTGCTGCCCGAGTTGACGAAGCTCGGGTAGCTGGTGCCAGGGCT
GGCGTTCGGGCTCAGCAACGACGGGCTCAACTGCTTCGAGCCCATCGACCACGGTCCCGA
 R  L  G  S  D  N  S  G  S  N  V  F  S  P  Y  S  T  G  P  S

       2590      2600      2610      2620      2630      2640
TCTAAGGCCCGGGTTTGCGCCCGAGCCAGCCGCGGCACTGCCGCTACCGGGGTTCGGGTT
AGATTCCGGGCCCAAACGCGGGCTCGGTCGGCGCCGTGACGGCGATGGCCCCAAGCCCAA
 R  L  G  P  N  A  G  S  G  A  A  A  S  G  S  G  P  N  P  N

       2650      2660      2670      2680      2690      2700
GCCTGAGTCCAGGCCGCCAACAGGAGCACTGGCCGGGGCGGCGACGGGCGTGTTGGTCAG
CGGACTCAGGTCCGGCGGTTGTCCTCGTGACCGGCCCCGCCGCTGCCCGCACAACCAGTC
 G  S  D  L  G  G  V  P  A  S  A  P  A  A  V  P  T  N  T  L

       2710      2720      2730      2740      2750      2760
GCCCGAGTTGAGGACGTTCGCCAGGCCGTGTTGGAGACCGCCCGTTGATCCGAGGGCGGA
CGGGCTCAACTCCTGCAAGCGGTCCGGCACAACCTCTGGCGGGCAACTAGGCTCCCGCCT
 G  S  N  L  V  N  A  L  G  H  Q  L  G  G  T  S  G  L  A  S

       2770      2780      2790      2800      2810      2820
GGCGAGGATGCCCGAACTCAAAGCCGCCGTGCTCATGCCGCCGGTGGCGTAGCCGGCGGA
CCGCTCCTACGGGCTTGAGTTTCGGCGGCACGAGTACGGCGGCCACCGCATCGGCCGCCT
 A  L  I  G  S  S  L  A  A  T  S  M  G  G  T  A  Y  G  A  S

       2830      2840      2850      2860      2870      2880
GCTGACCAAGGCCGCCTCCGAGCCAGCCGCGCTTCCTAAGGCGGCGTTTTGCATCCCCGC
CGACTGGTTCCGGCGGAGGCTCGGTCGGCGCGAAGGATTCCGCCGCAAAACGTAGGGGCG
 S  V  L  A  A  E  S  G  A  A  S  G  L  A  A  N  Q  M  G  A

       2890      2900      2910      2920      2930      2940
GTTCCAGAAGCTGGTGTTGAGGCTGCCTGCGCTGCCGAGGCCCGCGTTGATTGTCCCCGA
CAAGGTCTTCGACCACAACTCCGACGGACGCGACGGCTCCGGGCGCAACTAACAGGGGCT
 N  W  F  S  T  N  L  S  G  A  S  G  L  G  A  N  I  T  G  S
```

FIGURE 8 (CONT'D)

```
       2950      2960      2970      2980      2990      3000
GGTCCCGATGCCGCTGTTCAGGGAGCCCGAATTCCCGATGCCGATGTTTCCGCTGCCGGA
CCAGGGCTACGGCGACAAGTCCCTCGGGCTTAAGGGCTACGGCTACAAAGGCGACGGCCT
 T  G  I  G  S  N  L  S  G  S  N  G  I  G  I  N  G  S  G  S

       3010      3020      3030      3040      3050      3060
GTTGAATAAGCCGACGTTGCCGGTGCCCGAGTTCCCGAAGCCGATGTTGCCGCTACCCGA
CAACTTATTCGGCTGCAACGGCCACGGGCTCAAGGGCTTCGGCTACAACGGCGATGGGCT
 N  F  L  G  V  N  G  T  G  S  N  G  F  G  I  N  G  S  G  S

       3070      3080      3090      3100      3110      3120
GTTGAAGCCGCCGAAACCCATCTGGTGATCACCGGTGATCCCGAACCCGATATTCCCGCT
CAACTTCGGCGGCTTTGGGTAGACCACTAGTGGCCACTAGGGCTTGGGCTATAAGGGCGA
 N  F  G  G  F  G  M  Q  H  D  G  T  I  G  F  G  I  N  G  S

       3130      3140      3150      3160      3170      3180
ACCGGTGTTGCCGAAGCCGATATTCCCGTCGCCGAGGTTGCCGAGGCCCAGGTTGCCGCT
TGGCCACAACGGCTTCGGCTATAAGGGCAGCGGCTCCAACGGCTCCGGGTCCAACGGCGA
 G  T  N  G  F  G  I  N  G  D  G  L  N  G  L  G  L  N  G  S

       3190      3200      3210      3220      3230      3240
GCCGGTGTTGCCGCTGCCGATGTTGCCGGTGCCGGTGTTGCCGCTGCCGATGTTGTTGTT
CGGCCACAACGGCGACGGCTACAACGGCCACGGCCACAACGGCGACGGCTACAACAACAA
 G  T  N  G  S  G  I  N  G  T  G  T  N  G  S  G  I  N  N  N

       3250      3260      3270      3280      3290      3300
GCCGATGTTGTTGTTGCCGATGTTGTTGTTGCCGATGTTGCCGCTGCCGGTGTTGCCGAA
CGGCTACAACAACAACGGCTACAACAACAACGGCTACAACGGCGACGGCCACAACGGCTT
 G  I  N  N  N  G  I  N  N  N  G  I  N  G  S  G  T  N  G  F

       3310      3320      3330      3340      3350      3360
GCCCAGATTGATCTGGCCGTTCTTGCCGATGTCGATGCCGAGGTTCCGCAAGACCTGCTG
CGGGTCTAACTAGACCGGCAAGAACGGCTACAGCTACGGCTCCAAGGCGTTCTGGACGAC
 G  L  N  I  Q  G  N  K  G  I  D  I  G  L  N  R  L  V  Q  Q
```

FIGURE 8 (CONT'D)

```
        3370      3380      3390      3400      3410      3420
CCAGGGCGCCAGTTGTGCGACGGCCGCAGACGCATCGAAGTGGTAACCAGCCATCGCCGC
GGTCCCGCGGTCAACACGCTGCCGGCGTCTGCGTAGCTTCACCATTGGTCGGTAGCGGCG
 W  P  A  L  Q  A  V  A  A  S  A  D  F  H  Y  G  A  M  A  A

        3430      3440      3450      3460      3470      3480
CACGTCCAATGCCCACATTTGCTCGTATGCCGCCTCGACGTCCATGAGCGCCGGAGCGTT
GTGCAGGTTACGGGTGTAAACGAGCATACGGCGGAGCTGCAGGTACTCGCGGCCTCGCAA
 V  D  L  A  W  M  Q  E  Y  A  A  E  V  D  M  L  A  P  A  N

        3490      3500      3510      3520      3530      3540
CTGCCCAAACCAGTTCGTAGCTGCCAGCAGCTGCATCAGGCCACGATTGGCCGCTACCAC
GACGGGTTTGGTCAAGCATCGACGGTCGTCGACGTAGTCCGGTGCTAACCGGCGATGGTG
 Q  G  F  W  N  T  A  A  L  L  Q  M  L  G  R  N  A  A  V  V

        3550      3560      3570      3580      3590      3600
TGCCGGCTGCACGGTGGCCGCCAGCGCCGCCTCGAACGCGGTCGCTGTTGCCATGGCCTG
ACGGCCGACGTGCCACCGGCGGTCGCGGCGGAGCTTGCGCCAGCGACAACGGTACCGGAC
 A  P  Q  V  T  A  A  L  A  A  E  F  A  T  A  T  A  M  A  Q

        3610      3620      3630      3640      3650      3660
TGCGGCCGCTTGTTCCGCCTGCGCTGCCGCCGTGCTGAGCCAGGCTAGGTACTGGGTTGC
ACGCCGGCGAACAAGGCGGACGCGACGGCGGCACGACTCGGTCCGATCCATGACCCAACG
 A  A  A  Q  E  A  Q  A  A  A  T  S  L  W  A  L  Y  Q  T  A

        3670      3680      3690      3700      3710      3720
GACGGCCATCATCGCCGCCGCGGACGGACCCAGCCAGGCGCCACTAGTCAGTTCGGATGT
CTGCCGGTAGTAGCGGCGGCGCCTGCCTGGGTCGGTCCGCGGTGATCAGTCAAGCCTACA
 V  A  M  M  A  A  A  S  P  G  L  W  A  G  S  T  L  E  S  T

        3730      3740      3750      3760      3770      3780
GACGGAGCCAAGCGACGCTATTGACGCGAGCAATTCTTCGGCCAGCTCGCCCCAGGCGGT
CTGCCTCGGTTCGCTGCGATAACTGCGCTCGTTAAGAAGCCGGTCGAGCGGGGTCCGCCA
 V  S  G  L  S  A  I  S  A  L  L  E  E  A  L  E  G  W  A  T
```

FIGURE 8 (CONT'D)

```
       3790      3800      3810      3820      3830      3840
GGCCGCAGCAATTAGCGGTCCCGACCCGGGACCGGCAAACATCAGTGCCGAATTGATCTC
CCGGCGTCGTTAATCGCCAGGGCTGGGCCCTGGCCGTTTGTAGTCACGGCTTAACTAGAG
 A  A  A  I  L  P  G  S  G  P  G  A  F  M  L  A  S  N  I  E

       3850      3860      3870      3880      3890      3900
TGGCGGCAACCACGCAAAATGCGGGCTTGTCAGCCGATCCAACTTAACTGTCAGCGACCG
ACCGCCGTTGGTGCGTTTTACGCCCGAACAGTCGGCTAGGTTGAATTGACAGTCGCTGGC
 P  P  L  W  A  F  H  P  S  T  L  R  D  L  K  V  T  L  S  R

       3910      3920      3930      3940      3950      3960
TTGCCGTGGCGGTATCGGCACTTCAATACCACTCATCTTTGGGGTCATCTTTGGAGCGCC
AACGGCACCGCCATAGCCGTGAAGTTATGGTGAGTAGAAACCCCAGTAGAAACCTCGCGG
 Q  R  P  P  I  P  V  E  I  G  S  M  K  P  T  M  K  P  A  G

       3970      3980      3990      4000      4010      4020
CCTAGGAACCGCCAGCTTACCTAGTCCCGGGTAGGGGCCGACTGGCGGCCGGGATGCAGC
GGATCCTTGGCGGTCGAATGGATCAGGGCCCATCCCCGGCTGACCGCCGGCCCTACGTCG
 R  P  V  A  L  K  G  L  G  P  Y  P  G  V  P  P  R  S  A  A

       4030      4040      4050      4060      4070      4080
TGAGGGTCTGCCACCTGCCCCGTAATGTCGCTGGTATGGCAAGCACCGACGCCGCGGCCC
ACTCCCAGACGGTGGACGGGGCATTACAGCGACCATACCGTTCGTGGCTGCGGCGCCGGG
 S  P  R  G  G  A  G  Y  H  R  Q  Y  P  L  C  R  R  R  P  G

       4090      4100      4110      4120      4130      4140
AAGAGTTGCTCCGCGACGCGTTCACCCGGTTGATCGAACATGTCGACGAACTCACCGACG
TTCTCAACGAGGCGCTGCGCAAGTGGGCCAACTAGCTTGTACAGCTGCTTGAGTGGCTGC
 L  T  A  G  R  R  T  *

       4150      4160      4170      4180      4190      4200
GCCTCACCGACCAACTCGCCTGCTACCGCCCGACCCCCAGCGCCAACAGCATTGCGTGGC
CGGAGTGGCTGGTTGAGCGGACGATGGCGGGCTGGGGGTCGCGGTTGTCGTAACGCACCG
```

FIGURE 8 (CONT'D)

```
      4210      4220      4230      4240      4250      4280
TGCTCTGGCACAGCGCCCGGGTGCAGGATATACAGGTCGCCCATGTGGCCGGCGTGGAAG
ACGAGACCGTGTCGCGGGCCCACGTCCTATATGTCCAGCGGGTACACCGGCCGCACCTTC

      4270      4280      4290      4300      4310      4320
AGGTGTGGACCCGCGACGGTTGGGTGGACCGCTTTGGGTTAGATCTGCCGCGGCACGACA
TCCACACCTGGGCGCTGCCAACCCACCTGGCGAAACCCAATCTAGACGGCGCCGTGCTGT

      4330      4340      4350      4360      4370
CCGGATATGGACACCGTCCCGAGGATGTGGCGAAGGTACGGGCACCCGCCGACGGAATTC  3'
GGCCTATACCTGTGGCAGGGCTCCTACACCGCTTCCATGCCCGTGGGCGGCTGCCTTAAG  5'
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(57) Abstract

Mycobacterium tuberculosis genes encoding five immunologically relevant proteins have been isolated by systematically screening a lambda gt11 recombinant DNA expression library with a collection of murine monoclonal antibodies directed against protein antigens of this pathogen. One of the *M. tuberculosis* antigens, a 65kD protein, has been shown to have determinants common to *M. tuberculosis* and *M. leprae*. In addition, genes encoding proteins of other mycobacteria (*M. africanum, M. smegmatis, M. bovis* BCG and *M. avium*) have been isolated. Isolation and characterization of genes encoding major protein antigens of *M. tuberculosis* make it possible to develop reagents useful in the diagnosis, prevention and treatment of tuberculosis. They can be used, for example, in the development of skin tests, serodiagnostic tests and vaccines specific for tuberculosis.

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MYCOBACTERIUM TUBERCULOSIS GENES AND ENCODING PROTEIN ANTIGENS

Description

Background

Tuberculosis was the major cause of infectious mortality in Europe and the United States in the 19th and early 20th centuries. Dubos, R. and J. Dubos, The White Plague: Tuberculosis, Man and Society, Little Brown & Co., Boston, MA, (1952). Today, it remains a significant global health problem.

For example, in the United States there are over 20,000 new cases of tuberculosis diagnosed annually. In addition, the steadily declining incidence of tuberculosis evident in preceding years appears to have changed course, reaching a plateau in 1985 and showing an increase in the first half of 1986. Centers for Disease Control, Morbidity/Mortality, Weekly Report, 34:774 (1986); and Centers for Disease Control, Morbidity/Mortality, Weekly Report, 35:774 (1986).

Worldwide, tuberculosis remains widespread and constitutes a health problem of major proportions, particularly in developing countries. The World Health Organization estimates that there are ten million new cases of active tuberculosis per year and an annual mortality of approximately three

million. Joint International Union Against Tuberculosis and World Health Organization Study Group, *Tubercle*, *63*:157-169 (1982).

Tuberculosis is caused by *Mycobacterium* (*M.*) *tuberculosis* or *Mycobacterium* (*M.*) *bovis*, which are the 'tubercle bacilli' of the family *Mycobacteriaceae*. *M. bovis* is a species which causes tuberculosis in cattle and is transmissible to humans and other animals, in whom it causes tuberculosis. At present, nearly all tuberculosis is caused by respiratory infection with *M. tuberculosis*. Infection may be asymptomatic in some, but in other individuals, it produces pulmonary lesions which lead to severe debilitation or death. Resistance to tuberculosis is provided by cell-mediated immune mechanisms.

Mycobacteria are aerobic, acid-fast, non-spore-forming, non-motile bacili with high lipid contents and slow generation times. *M. leprae* is the etiologic agent of leprosy and, among the other mycobacteria, the only major pathogen. Bloom, B.R. and T. Godal, *Review of Infectious Diseases*, *5*:765-780 (1983). However, other mycobacterial species are capable of causing disease. Wallace, R.J. *et.al.*, *Review of Infectious Diseases*, *5*:657-679 (1984). *M.avium*, for example, causes tuberculosis in fowl and in other birds. Members of the *M. Avium-intracellularae* complex have become important pathogens among individuals with acquired immunodeficiency syndrome (AIDS). Certain groups of

individuals with AIDS have a markedly increased incidence of tuberculosis as well. Pitchenik, A.E. et. al., Annals of Internal Medicine, 101:641-645 (1984).

Diagnostic and immunoprophylatic measures for mycobacterial diseases have changed little in the past half century. Tuberculin, developed by Koch as a cure for tuberculosis in the late 1800s, is an M. tuberculosis filtrate of complex and poorly-defined composition. It is used as a skin test antigen to detect prior exposure to the bacillus. Enrichment of the protein fraction of this material in the 1930's produced the purified protein derivative (PPD) which is still used to diagnose exposure to tuberculosis. Seibert, F.M. et.al., American Review of Tuberculosis, 30(Suppl.):705-778 (1934). Its usefulness is limited, however, by its lack of specificity and its inability to distinguish active disease from prior sensitization by contact with M. tuberculosis or cross-sensitization to other mycobacteria. Young, R.A. and R.W. Davis, Proceedings of the National Academy of Sciences, USA, 80:194-1198 (1983).

Bacille Calmette Guerin (BCG), an avirulent strain of M. bovis, has been used widely as a live vaccine against tuberculosis for over 50 years. Calmette, A., C. et.al., Bulletin of the Academy of

Medicine Paris, 91:787-796 (1924). During that time, numerous studies have shown that BCG has protective efficacy against tuberculosis. These studies are reviewed by F. Luelmo in American Review of Respiratory Diseases, 125(pt. 2):70-72 (1982). However, more recently, a major trial of BCG in India indicated that such a vaccine was not protective against tuberculosis in this setting. World Health Organization WHO Technical Report Series, 651 (1980). Presently available approaches to diagnosing, preventing and treating tuberculosis are limited in their effectiveness and must be improved if a solution is to be found for the important public health problem tuberculosis represents worldwide.

Summary of the Invention

The present invention is based on the isolation of genes encoding immunogenic protein antigens of the tubercle bacillus *Mycobacterium tuberculosis* (*M. tuberculosis*). Genes encoding such protein antigens have been isolated from a recombinant DNA expression library of *M. tuberculosis* DNA. Genes encoding proteins of four additional mycobacteria have also been isolated and restriction maps produced.

In particular, genes encoding five immunodominant protein antigens of the tuberculosis bacillus (i.e., those *M. tuberculosis* proteins of molecular weight 12,000 daltons (12kD), 14kD, 19kD, 65kD and 71kD have been isolated by probing a lambda gt11 expression library of *M. tuberculosis* DNA with